Coulochem® III Analyst® Driver

Version 1.0

SOFTWARE APPLICATION MANUAL

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1. Introduction

1.1 Overview

This manual describes the basic setup and operation of ESA Biosciences' Coulochem III Electrochemical Detector for use as an integrated device within Applied Biosystems / MDS Sciex Analyst Software. The Analyst program enables you to control ABI's mass spectrometers and other devices, such as autosamplers, pumps, column ovens, and valves. Once installed, the Coulochem III "plug-in" driver is provided as a companion Software Application in the Available Devices list within Analyst. This allows you to configure and control the Coulochem III detector within the Analyst interface in a regulated environment using electronic records and the associated features in Analyst software.

The Coulochem III driver application supports control of the Coulochem III device by serial RS232 or USB communications and uses an analog-to-digital converter (ADC) supported by Analyst for data acquisition.

Documentation for the Coulochem III detector includes:

- ***Coulochem III User's Guide*** (Part Number 70-4789) - designed to provide sufficient information to allow the analyst to collect analytical data in DC mode (DC Mode requires the use of the *DC Potentiostat Board*).
- ***Coulochem III Reference Manual*** (Part Number 70-4779) - provides a detailed discussion on the care and use of the detector, including method development, installation, maintenance and advanced topics such as the use of Pulse, Scan and Redox Modes (Pulse and Scan Modes require the *Pulse/Scan Potentiostat Board*).

1.2 Contents of the Coulochem III Analyst Driver Software Application Manual

This manual includes the following material:

- ***System Requirements and Installation*** (Chapter 2) – describes the minimum computer system requirements for operation of the driver software, as well as the procedure for installing the driver and verifying the installation.
- ***Starting and Configuring the Driver*** (Chapter 3) – describes the procedure for starting the driver software and adjusting the configuration parameters to meet the system arrangement.
- ***Creating and Modifying Acquisition Methods*** (Chapter 4) – describes the procedure for creating and modifying the method files describing the set of

parameters and series of events that make up the acquisition method. This chapter also includes a description of how to add the Coulochem III driver to an Analyst Hardware Profile.

- ***Basic Coulochem III Driver Functions*** (Chapter 5) – provides a description of driver functions that can be accessed while the Coulochem III detector is not actively running an acquisition method.

- ***Acquiring Data with the Coulochem III Driver*** (Chapter 6) – describes the procedure for performing a sample data acquisition run with the Analyst program and Coulochem III driver.

- ***Application Examples*** (Chapter 7) – provides some useful examples of how the Coulochem III detector can be combined with a mass spectrometer to detect and identify various compounds of interest.

- ***Updating the Coulochem III Firmware*** (Appendix A) – describes the procedure for updating the Coulochem III firmware.

- ***Parameter Ranges and Default Values*** (Appendix B) – Tabulates all of the program parameters used in the Coulochem III driver, along with their range of values and default values.

1.3 Getting Help

The Coulochem III driver provides many ways to obtain help about a particular parameter, topic or feature while using the program. These include Help buttons, a Help Navigator tool, Help menu items and access to the entire manual as an Adobe PDF file.

1.3.1 Help Buttons and the Coulochem III Help File

Most windows and dialog boxes in the driver program provide a **Help** button for obtaining help. Clicking the **Help** button automatically opens the Coulochem III Analyst Driver Help file to a topic that is relevant to the window or dialog box. For example, clicking the **Help** button on the main driver window while the Pre-Run tab is visible opens the Help file to the Pre-Run Tab topic.

1.3.2 Help Navigator Tool

Each window or dialog box in the driver program also provides a Help Navigator tool for obtaining help. The tool is enabled by clicking the button in the upper right corner of the window or dialog box. When activated, the cursor changes to a question mark. Clicking on any control (i.e., any edit box, check box, selection box or icon) produces a brief help statement describing the function of the control. Figure 1.1 shows both the Help Navigator button (upper right corner) and the help

cursor (over the **Auto Zero** button), and Figure 1.2 shows the help statement that appears when the Help Navigator cursor is clicked over the **Apply Settings** button.

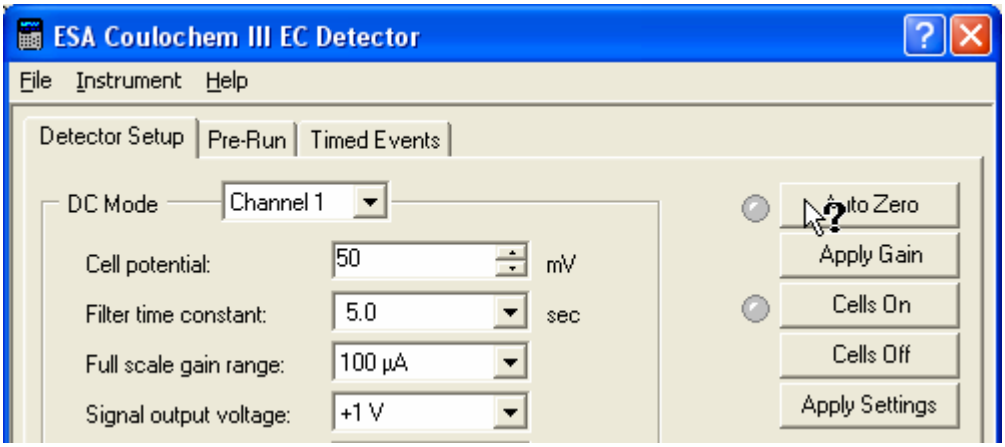


Figure 1.1. Help Navigator Icon (upper right) and Cursor.

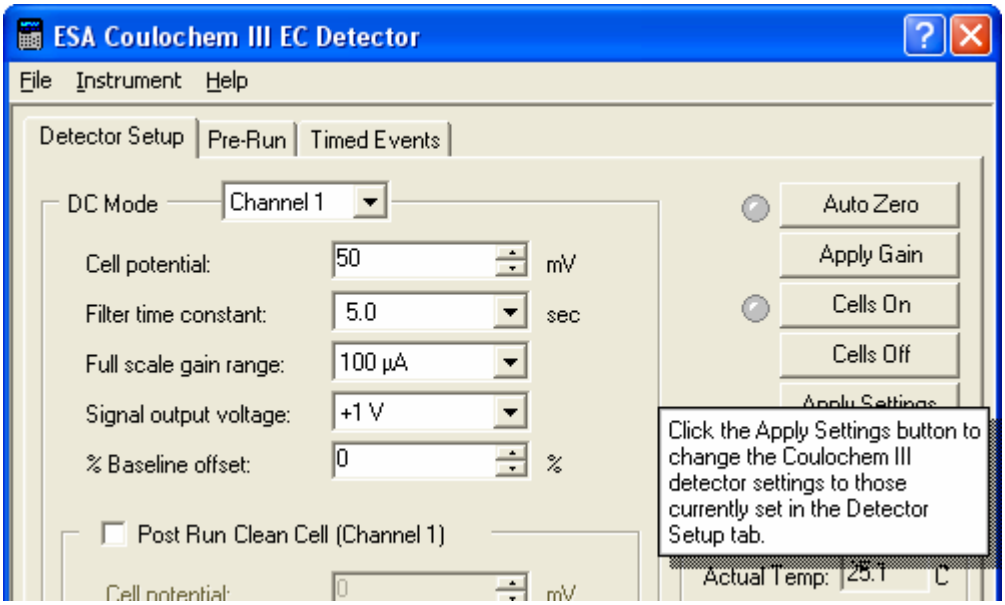


Figure 1.2. Help Navigator Text for the Apply Settings button.

1.3.3 Help Menu

The **Help** menu provides direct access to the entire Coulochem III Analyst Driver Help file. Select **Help > Contents** to open the help file to its table of contents. Select **Help > Index** to open the help file to its index of terms.

The Coulochem III Analyst Driver Help file provides all of the text of this manual in an easy to navigate hypertext form.

You can use the Search feature to search for all information about a particular term or terms, e.g. “guard cell”.

1.3.4 Entire Manual in Adobe PDF Format

Select **Help > Open Manual in PDF Format** to open the Adobe PDF version of this Software Manual. (Adobe Reader or Adobe Acrobat is required.)

2. System Requirements and Installation

2.1 System Requirements

The Coulochem III driver application has been designed and tested to operate under the minimum hardware and software specifications listed below.

- Microsoft® Windows®-based PC with Intel Pentium 4 processor operating at 1.5 GHz.
- Microsoft® Windows® XP Professional with Service Pack 2 (SP2) installed.
- Installation of the Microsoft® .NET® runtime (added automatically during program installation, if not already present).
- 1 GB RAM.
- 40 GB hard drive (less than 1 GB available space required for program installation).
- Available serial port or USB port for Coulochem III detector connection.
- Monitor with 1024 x 768 minimum pixel resolution.
- Two-button mouse.
- Analyst software, version 1.4.1 or later (including April and June, 2005 hotfixes).

If using Analyst version 1.4.1, the Analyst hotfixes for April and June, 2005 must be installed in order for Analyst to work with the Coulochem III driver. If necessary, check with your Applied Biosystems / MDS Sciex representative for information on how to obtain and implement these hotfixes.

2.2 Additional Hardware Requirements

- RS232 serial cable, ESA P/N 70-1743. This cable is not required if connecting through the USB port.
- Coulochem III firmware version 3.07 or later. (See Appendix A for instructions on updating the firmware.)
- Standard type A to B USB cable, ESA P/N 70-5713. Not required if connecting through the RS232 port, but required if updating the firmware becomes necessary.

- (Optional) Analyst-supported ADC – for analog signal output to Analyst. Obtained from National Instruments; see your ABI representative.

Either an RS232 cable or a USB cable is required for connecting to the detector.

2.3 Installing the Coulochem III Analyst Driver

To install the Coulochem III Analyst driver on your PC, perform the following steps.

1. Ensure that the Coulochem III detector is off or disconnected. (If on, the detector could interfere with the installation of the USB driver.)

2. Close all running software applications. Then insert the Coulochem III Analyst Driver CD into your CD-ROM drive. The installation program should start automatically (unless CD auto-run is disabled on the PC). If it does not start automatically, click **Start > Run**, then type *drive*:\setup (where *drive* is the drive letter of your CD-ROM drive, e.g. D:\setup), then click **OK**. The InstallShield Wizard should appear, as in Figure 2.1 below.

Coulochem III Analyst Driver - InstallShield Wizard
esa
MAGELLAN BIOSCIENCES
Welcome to the InstallShield Wizard for Coulochem III Analyst Driver
The InstallShield® Wizard will install Coulochem III Analyst Driver on your computer. To continue, click Next.
< Back
Next >
Cancel

Figure 2.1. Installation Introductory Page.

3. Click the **Next** button. This will display the License Agreement.

4. Read the License Agreement and print a copy, if desired, by clicking the **Print** button. If you agree to the terms of the License Agreement, select the **I Accept...** button and click **Next**.

5. When the Ready to Install dialog appears, click **Install** to start the driver installation process.

6. The installer will add the Coulochem III Analyst driver files to the folder C:\Program Files\ESA Biosciences\Coulochem III Analyst Driver. It will then automatically install the USB driver for the Coulochem III detector.

7. When finished, click the **Finish** button.

8. Remove the installation CD and store it in a safe place.

After installation, an icon will appear on the desktop for starting the Coulochem III driver (with or without first starting Analyst), and a menu entry will appear in the **Start > All Programs** menu under ESA Biosciences.

2.4 Hardware Configuration

The Coulochem III detector can be customized to accommodate a wide variety of analytical needs. The 'Coulochem® III for MS' is the standard configuration for use with ABI's mass spectrometers. This platform allows the use of the Coulochem III module to control electrochemical (EC) cells with fixed (DC) or dynamic (pulsed) potential waveforms. Cells can be used as reactors in series upstream of the MS ion source and/or as detectors in parallel (via flow split) with MS. Serial EC/LC-MS configurations allow MS study of EC reactions and products while parallel EC with MS provides complementary and highly sensitive detection of redox active species. Some examples of these applications are provided in Chapter 7 of this manual. For additional detail please contact your ESA Biosciences representative.

The Coulochem® III for MS (ESA P/N 70-6045A) includes the Controller with DC and Pulse Mode capability, the Model 5021A EC cell (reactor cell with ca 16µL swept volume) Model 5030 EC cell (reactor cell with ca 3µL swept volume), cables and Ground Decoupling Kit. Cells and optional accessories include:

Model 5021A Conditioning (Reactor) Cell ..... ESA P/N 70-6068
Model 5030 Low Volume Reactor Cell ..... ESA P/N 70-6069
Model 5010A Dual Analytical Cell ..... ESA P/N 70-5560
Model 5040 Analytical Cell with Au Target ..... ESA P/N 55-0185
Adjustable Analytical Flow Splitter ..... ESA P/N 70-6337
Thermally Controlled Cell Organizer Module ..... ESA P/N 70-5499TA

To ensure proper grounding, it is essential that a Ground Decoupler Kit (ESA P/N 70-5971) be used for connections between the mass spectrometer and the electrochemical cell(s) and modules.

2.5 Connecting the Communications Cables

The Coulochem III detector can be connected to the PC by either an RS232 serial cable or a USB cable. (USB is preferred, and a USB connection is required in order to perform firmware updates – see Appendix A.)

If using a serial cable, connect one end of the cable to the RS232 Remote port on the Coulochem III detector (Figure 2.2) and the other on an available COM port on the PC.

If using a USB cable, connect the small end of the cable to the USB Remote port on the Coulochem III detector (Figure 2.2) and the wide end to an available USB port on the PC.

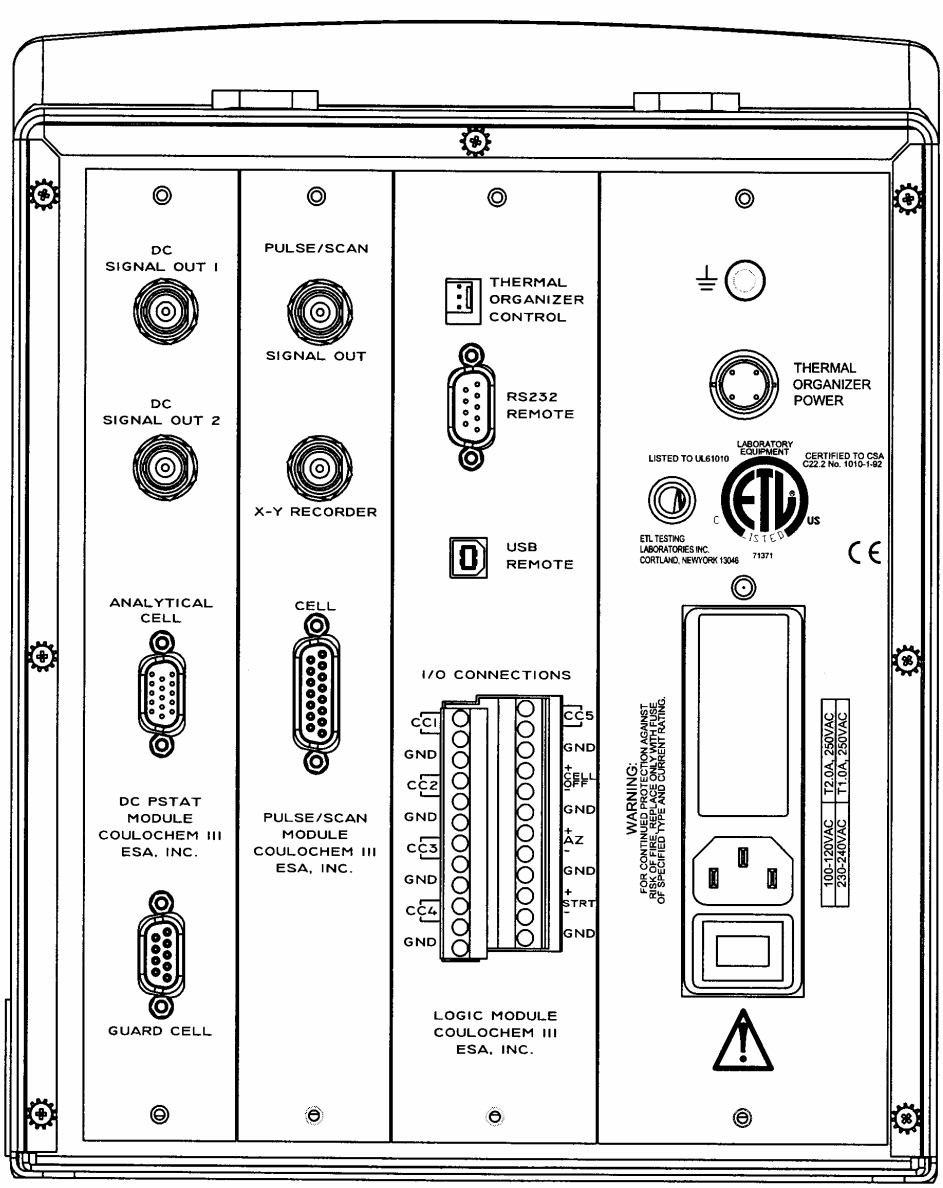


Figure 2.2. Coulochem® III Back Panel.

2.6 Verifying the System

To operate properly, the Analyst program must be able to locate and communicate with the Coulochem III driver (companion software). Proper installation of both the Analyst program (with any required hotfixes) and the Coulochem III driver is essential in order for this communication to occur. (If necessary, check with your Applied Biosystems / MDS Sciex representative to ensure that you have the required hotfixes.)

To verify the installation, perform the following steps:

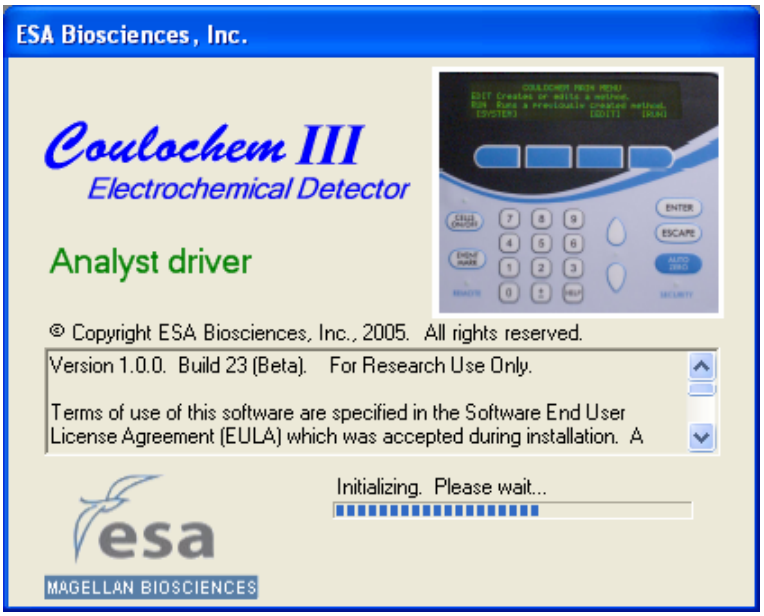
1. Turn the Coulochem III detector on and allow ample time for it to complete its startup routine.

2. Start the Analyst program. If required, enter a login name and password (required when running in Mixed mode).

3. Ensure that the Analyst *Navigation Bar* is visible. (Select **View** > **Navigation Bar** if not.)

4. Verify that the item **Coulochem III Detector** appears in the *Companion Software* section at the bottom of the Navigation Bar.

5. Double-click on the **Coulochem III Detector** item in the Navigation Bar. This should start the Coulochem III driver, which will display its introductory splash screen, as shown in Figure 2.3 below. (If running Analyst in Mixed mode, it will be necessary to log on again prior to seeing the splash screen.)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Coulochem III
Electrochemical Detector
Analyst driver
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Terms of use of this software are specified in the Software End User License Agreement (EULA) which was accepted during installation. A
Initializing. Please wait...
esa
MAGELLAN BIOSCIENCES

Figure 2.3. Coulochem III Driver Introductory "Splash" Screen.

6. After a few seconds the main driver window will appear.

7. If the Coulochem III detector is connected to the PC using a USB cable, a message box like the following may appear:

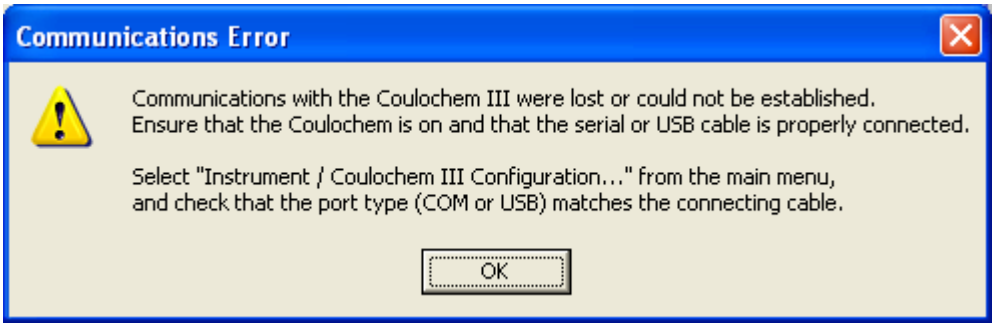

Communications Error
Communications with the Coulochem III were lost or could not be established.
Ensure that the Coulochem is on and that the serial or USB cable is properly connected.
Select "Instrument / Coulochem III Configuration..." from the main menu,
and check that the port type (COM or USB) matches the connecting cable.
OK

Figure 2.4. Coulochem III Driver Communications Warning.

If this occurs, click **OK**. The message appears because the initial default configuration assumes that the detector is connected through the serial port. This situation will be corrected during configuration of the driver in a later section.

8. Confirm that the term **Logged onto Analyst** appears, in a dark green background, in the Acquisition section at the bottom of the driver window.

9. Confirm that the term **Connected to CcIII** appears, also in a dark green background. If the term **Not connected to CcIII** appears (in a bright red background), check the serial cable connection. (If connected through a USB port, ignore the warning; the situation will be corrected in a later section.)

3. Starting and Configuring the Driver

3.1 Overview

Prior to using the Coulochem III Analyst driver to collect data, the driver needs to be configured to match the actual Coulochem III detector configuration (including the connection port). The detector connections were considered in Chapter 2. In this chapter, we will ensure that the Coulochem III Analyst driver is configured to match the detector configuration.

This chapter discusses how to set up the initial configuration of the driver, including the port connection and hardware (cell) options. Once configured, changes to the configuration should seldom be needed.

3.2 Starting the Coulochem III Analyst Driver

1. Turn on the Coulochem III detector and allow ample time for it to complete its startup routine.

2. Start the Analyst program. If required, enter a login name and password (required when running in Mixed mode).

3. Ensure that the Navigation Bar is visible. (Select **View** > **Navigation Bar** if not.)

4. Double-click on the **Coulochem III Detector** item in the Navigation Bar. This should start the Coulochem III Analyst driver, which will display its splash screen, as shown in the previous chapter. (If running Analyst in Mixed mode, it will be necessary to log on again prior to seeing the splash screen.)

5. After a few seconds the main driver window will appear.

6. If a message box like the one shown in Figure 2.4 above appears, click **OK**. It may be possible to correct the problem in Section 3.3, but observe the warning note at the end of this section.

As soon as successful connections are made with both the Analyst program and the Coulochem III detector, and the Coulochem III detector has completed its self-test sequence, the Status area at the bottom of the driver window displays the connection status states in green, as in Figure 3.1.

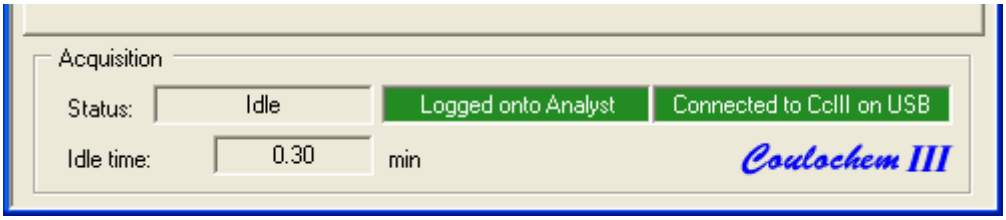


Figure 3.1. Status Bar Showing Successful Connections.

During the self-test sequence, the Coulochem III connection status appears in a yellow field, as in Figure 3.2.

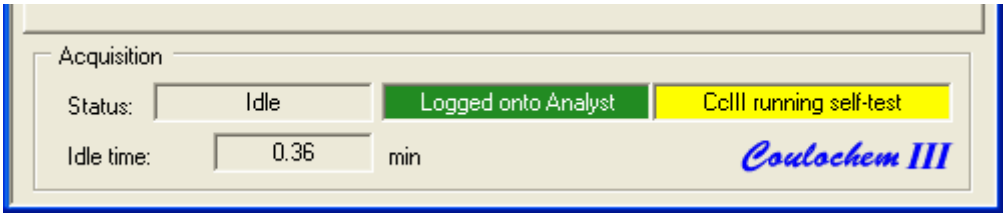


Figure 3.2. Status Bar During Coulochem III Self-Test.

The appearance of either of the above status conditions indicates a successful connection with the Analyst program and the Coulochem III. If either the Analyst or Coulochem III connection fails for any reason, the failure is indicated by a red background, as in Figure 3.3.

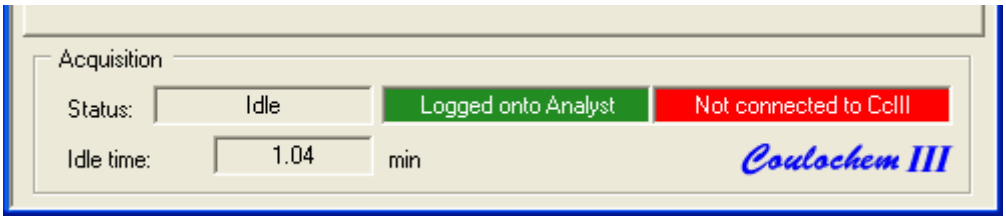


Figure 3.3. Status Bar Indicating a Failed Coulochem III Connection.

WARNING: After the communications port has been properly configured, the Communications Error message box (Figure 2.4) should no longer appear when starting up the driver. If it should appear at a later time, the warning should not be disregarded, since it indicates a possible problem with communications between the detector and the PC. Note that the problem could be as simple as forgetting to turn on the Coulochem III detector.

3.3 Accessing the Coulochem III Driver Window

The Coulochem III driver works alongside the Analyst program, so normally both programs are open at the same time. This will often cause the driver window to be "hidden" behind the Analyst program. There are many simple ways to make the driver visible when this happens, including any of the following:

- Click the **ESA Coulochem III** button on the Windows task bar at the bottom of the display.
- Double-click the **Coulochem III Detector** item in Analyst's Navigation Bar. (This will not open another instance of the driver.)
- Press **Alt-Tab** from the keyboard and select the driver.

NOTE: Although the normal procedure is to start Analyst first and then start the Coulochem III driver, it is possible to run the Coulochem III Analyst driver without running Analyst first, by double-clicking the Coulochem III Analyst Driver icon on the desktop or selecting Coulochem III Analyst Driver from the ***Start > All Programs*** *menu under ESA Biosciences. When the driver appears to be running without Analyst, it is actually still connected to Analyst through a Windows service. This service allows changes to be made to the driver configuration without having to start the Analyst program. It is also possible to make changes to acquisition methods this way, without having to open the Analyst program. It is not possible, however, to create new methods without Analyst. All acquisition methods must be initiated in Analyst, as explained in subsequent sections.*

3.4 Configuring the Coulochem III Analyst Driver

The Coulochem III can be configured in either DC or pulsed mode. In DC mode, 1 or 2 channels can be utilized, along with an optional guard cell and thermal organizer. Operation in pulsed mode uses a single channel and does not use the guard cell.

The driver must be configured to reflect actual hardware as well as experimental preferences prior to use with the Analyst program for data acquisition.

1. From the driver menu, select **Instrument > Coulochem III Configuration**.

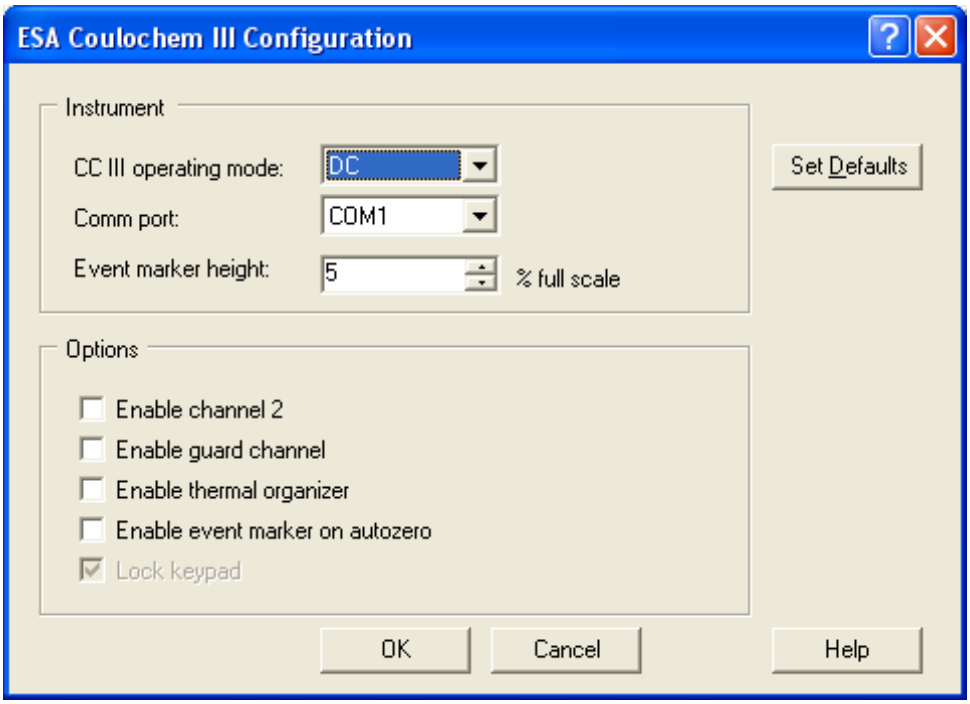


Figure 3.4. The Coulochem III Configuration Dialog

2. From the configuration dialog, Figure 3.4, select the appropriate options for each of the following parameters:

   - *CC III operating mode*
     Select either **DC** or **Pulse** mode.

   - *Comm port*
     Select **USB** if connected to the Coulochem III through an available USB port.

     If connected though a serial port, select either **COM1**, **COM2**, **COM3** or **COM4**, depending on which ports are available. (If unsure, start with COM1. If this causes problems later, return to the configuration and try COM2, COM3

and COM4 in order.)

- *Event marker height*
  During the course of a data acquisition run, the user may specify that a marker be placed in the acquisition data to designate a particular event, such as an autozero. The event marker height is the height of this marker, designated as a percent of the full scale data output range.

  Select a value between **-20%** and **+20%** full scale.

- *Enable channel 2*
  Check this box if the Coulochem III detector has been fitted with a second channel cell *and* you wish to use the cell in your next (first) acquisition. Most applications that use an EC cell primarily as a reactor typically only require a single channel (channel 1). Many applications of EC for detection and quantitation use dual EC cells to obtain higher selectivity, for example through oxidation-reduction or oxidative screening.

- *Enable guard channel*
  Check this box if the Coulochem III detector has been fitted with a guard cell *and* you wish to use the guard channel in your next (first) acquisition. The signal from the guard channel cannot be monitored using an ADC.

- *Enable thermal organizer*
  Check this box if the Coulochem III detector has been fitted with an optional thermal organizer (ESA P/N 70-5499T) and you wish to display the thermal organizer properties in the main driver window. (Activation of the organizer is controlled in the main driver window.)

- *Enable event marker on autozero*
  Check this box if you would like to have the driver automatically create a marker in the acquisition data whenever an autozero event occurs. The autozero marker height is determined by the *Event marker height* set above.

- *Lock keypad*
  This box is normally checked, and is not accessible to the user.

The **Set Defaults** button can be clicked at any time to set all values to their hard default values. (See *Appendix B* for a table of all user-accessible values, along with default and range values.)

3. Click **OK** to accept the changes made to the configuration.

4. If a change was made to the *Comm port* value, ensure that the connection status in the Acquisition section of the driver window now indicates **Connected to CcIII on *Port***, where *Port* is either USB or one of the COM ports. If the connection status appears as

Not connected to CcIII (in a bright red background), try one of the following to fix the problem:

a. Ensure that the Coulochem III detector is on.
b. Ensure that the connection (USB or serial cable) is secure.
c. If using a serial connection, try another COM port in the configuration.
d. If using a serial connection, check the serial port settings on the Coulochem III detector. To do this, perform the following steps:
    i. Exit the Coulochem III Analyst driver by selecting **File > Exit** from the menu.
    ii. From the main menu of the Coulochem III detector (COULOCHEM MAIN MENU visible on the display), press the SYSTEM button. This should activate the SYSTEM SETUP MENU on the display. (If the SYSTEM button is inactive, it may be necessary to turn the Coulochem III off and back on again. Then repeat and continue from this step.)
    iii. Press the NEXT button.
    iv. Press the DEFAULT button.
    v. Press the NEXT button.

NOTE: When properly configured, both the **Logged onto Analyst** and **Connected to CcIII** indicators should appear on a dark green background, as described in Section 3.2. An improper connection is indicated by a bright red background.

4. Creating and Modifying Acquisition Methods

4.1 Overview

An *acquisition method* consists of a set of parameters that describe the detector environment in which data acquisition is to occur as well as the duration and sequence of events that are to occur during the acquisition.

In a setting where both mass spectrometry and electrochemical detection are utilized, acquisition method parameters must be assembled for both instrument environments. The Analyst program manages all aspects of the mass spectrometry while the Coulochem III driver manages all aspects of electrochemical detection, receiving status updates on the execution of the mass spectrometer (MS), for synchronization. Despite this separation of instrument environments, all acquisition parameters are consolidated into a single method file (an Analyst *.dam), which is managed entirely by the Analyst program. This necessitates a somewhat strict protocol by which the MS and electrochemical detection method details are to be entered in a new method file, specifically:

a. Create a *Hardware Configuration Profile* (a "profile") in Analyst. This profile specifies all of the MS instrument parameters as well as basic properties of connected devices like pumps and autosamplers. The profile also provides the ability to specify that a Coulochem III detector is connected to the system.

   Once configured, the same profile can be used over and over again, until it is necessary to change basic instrument or device parameters. Multiple profiles can be created, stored and reused, but only one profile may be active at a time.

b. Build an acquisition method within Analyst. This method initially will contain all parameters to be used for MS control. The method is saved as a *.dam file by Analyst.

c. Build a Coulochem III method. This method details the parameters to be used, and activities to be run, on the Coulochem III detector. Coulochem III method parameters can only be set within the Coulochem III Analyst driver.

d. Attach the Coulochem III method data to the Analyst method data file. Without this step, the Coulochem III method would not be known by Analyst or available to it. *Attaching the Coulochem III method data to the Analyst *.dam file also allows the parameter data to be managed under the control of the Analyst program's secure filing system.*

Other configuration and method data, such as data for pumps and autosamplers, may be communicated to Analyst through their respective drivers.

The sections below describe each of these steps in detail.

4.2 Creating and Activating a Profile

A profile specifies all mass spectrometer instrument parameters as well as basic properties of connected devices like pumps, autosamplers, the Coulochem III detector and the A/D converter.

The instrument profile for a typical metabolite identification study (see section 7) may include a binary pump, autosampler, Coulochem III module and mass spectrometer. The Coulochem III module may be used to control a pre-column EC reactor cell. For a given parent compound, a series of injections is made, each with the cell set at a different applied potential. EC oxidation or reduction products are then separated chromatographically and characterized by mass spectrometry. The signal (current or charge) produced from the EC reactions may also be simultaneously monitored using the ADC.

The basic steps for creating a profile that includes the Coulochem III detector and ADC are:

1. From Analyst, double-click the **Hardware Configuration** item in the Navigation Bar. This opens Analyst's Hardware Configuration Editor dialog.

2. Click the **New Profile** button, to open Analyst's Create New Hardware Profile dialog.

3. Click the **Add Device** button.

4. Add a Mass Spectrometer device to the profile, as well as any pumps, ovens, autosamplers and other devices that you may require, by selecting from the *Device Type* drop list, then the *Devices* list and clicking **OK**. Refer to the Analyst program manual for specific details on adding and setting up these devices. (The Coulochem III module and ADC card additions are described in the next steps.)

5. Add the Coulochem III detector to the profile by clicking the **Add Device** button and then selecting **Software Application** from the *Device Type* drop list. Highlight Software Application from the *Devices* list and click **OK**. Figure 4.1 shows the Create New Hardware Profile dialog for a simple profile just after adding the Coulochem III driver as a Software Application.

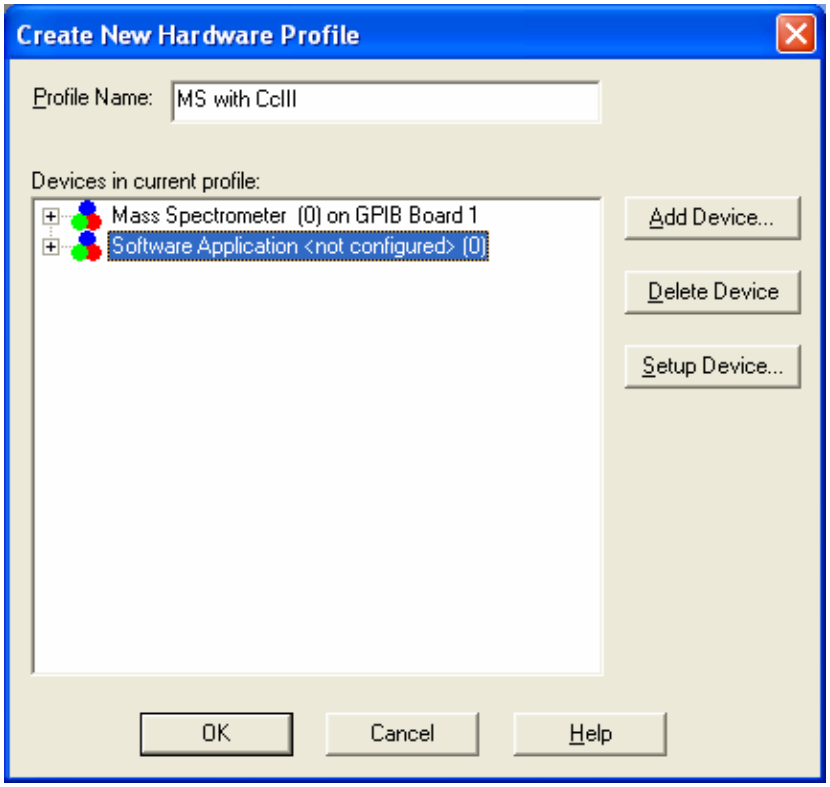


Figure 4.1. Hardware Profile with Coulochem III Detector (Unconfigured)

6. Highlight the Software Application text, then click the **Setup Device** button and select (highlight) Coulochem III Detector from the list of software applications in the Software Application Settings dialog, Figure 4.2.

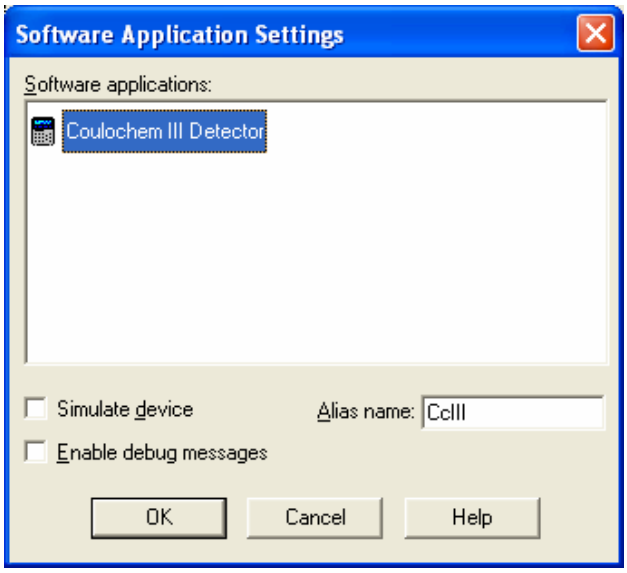


Figure 4.2. Software Applications Settings Dialog

7. Enter "CcIII", or something similar, as the alias name, then click **OK**.

8. If an ADC connection has been implemented between the Coulochem III detector and the PC, for collecting analog data from the Coulochem III detector, click the **Add Device** button again to configure the ADC. If an analog connection will not be used, continue at step 12 below.

9. Select A/D Converter from the *Device Type* drop list, then highlight A/D Converter NIDAQ16 (or the particular ADC to be used), and click **OK**.

10. Highlight the A/D Converter chosen and click **Setup Device**. The correct settings for the NIDAQ16 converter (NI 6032E PCI board) are illustrated in Figure 4.3 below for a 2-channel system. Note that each channel is configured separately. If 2 channels are to be used, be sure to configure the second channel by selecting **2** as the Channel No. and ensuring that the *Channel Connected* checkbox is checked for channel 2 (as in the figure).

    Note that for the NI 6032E PCI board, the *Polarity / Range* must be set to **-10.0V to +10.0V**, and the *Mode* must be set to **Referenced Single Ended**.

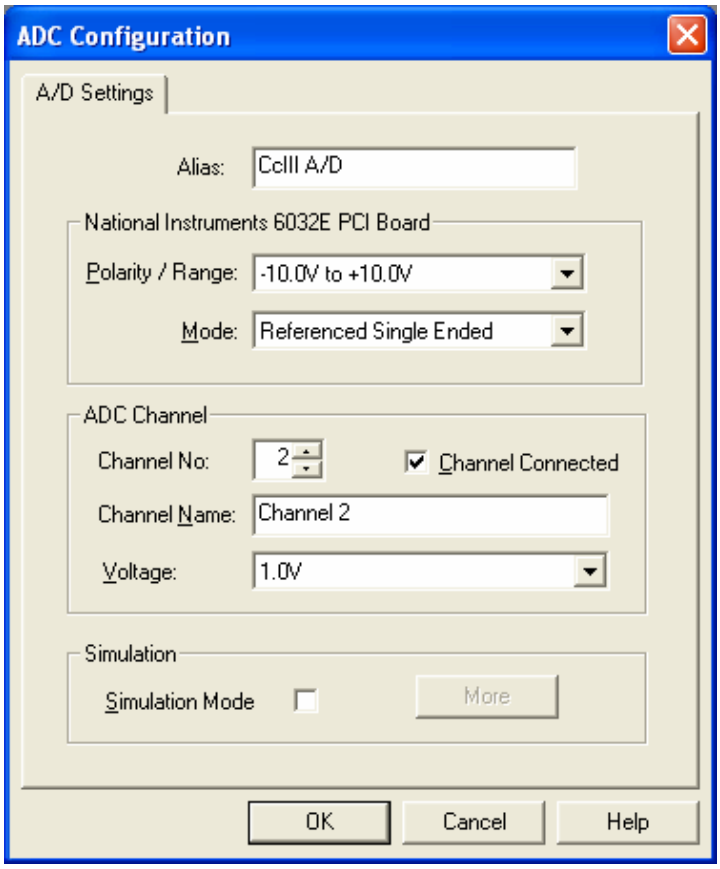


Figure 4.3. ADC Configuration for the NI 6032E PCI Board

11. Click **OK** to close the Create New Hardware Profile dialog.

12. Enter a profile name in the Profile Name edit box, then click **OK**.

    A profile's instrument components can be examined by clicking the small [+] box to the left of the profile name. Figure 4.4 shows the profile "MS with CcIII and ADC" configured with the MS, Coulochem III Detector and A/D Converter. Once configured, the same profile can be used over and over again, and profiles may be modified to accommodate changes in devices connected within a profile. Multiple profiles can be created, stored and reused, but only one profile may be active at a time.

13. Prior to building or using an acquisition method, a profile must be activated. Activate the newly created profile by selecting (highlighting) it and then clicking the **Activate Profile** button.

    Since the Coulochem III device is a part of the new profile, the Coulochem III driver should open soon after clicking the **Activate Profile** button. Allow the driver to finish its splash screen sequence, then click its Minimize button (upper right corner) to minimize its window to the Windows task bar.

14. Click **Close** to close the Hardware Configuration Editor dialog.

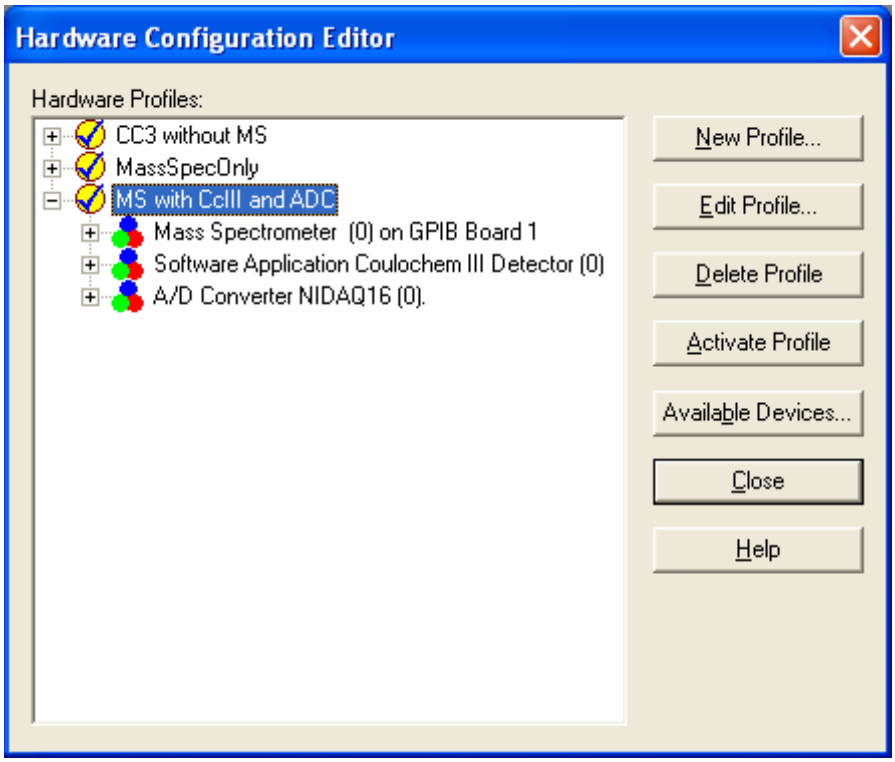


Figure 4.4. Example Configuration "MS with CcIII and ADC".

4.3 Building an Analyst Acquisition Method

All acquisition methods must be initiated in Analyst. Begin building a method by double-clicking the **Build Acquisition Method** item in the *Acquire* section of the Analyst Navigation Bar.

Set the parameters for the mass spectrometer according to the requirements of the experimental run. (Refer to the Analyst and MS manuals).

To set the A/D Converter properties, click on the A/D Converter item in the left panel.

1. In the Analog/Digital Converter Properties tab, adjust the *Rate* value to the desired sampling rate.

2. Ensure that a check appears in the checkbox corresponding to each channel for which you wish to have Analyst collect and store data.

3. Adjust the *Interpreted Value @ Full Scale* and *Interpreted Unit* values for each channel as required.

Note that clicking on the Coulochem III Detector item in the left panel displays an empty Software Application Properties tab. After the Coulochem III method has been configured, in the next section, this tab will display all Coulochem III method-related values.

Save and close the method, using a file name that will make it easy to remember the method's purpose or content.

4.4 Building a Coulochem III Method and Attaching to Analyst

Display the Coulochem III driver by clicking the **ESA Coulochem III** task button on the Windows task bar. If the driver does not appear on the task bar, restart it by double-clicking **Coulochem III Detector** in Analyst's Navigation Bar.

The Coulochem III driver window displays its method parameters in three different tabs: Detector Setup, Pre-Run, and Timed Events. In addition, if a thermal organizer is attached to the Coulochem III module and indicated in the configuration setup, controls for activating the organizer and for setting its setpoint temperature are displayed on the window.

Note: All of the method parameters and their ranges and default values are tabulated in Appendix B.

4.4.1 Detector Setup Tab

The Detector Setup tab displays the parameter settings for either **DC mode** or **Pulse mode** (depending on the configuration set up earlier).

4.4.1.1 DC Mode

DC mode method parameters are illustrated in Figure 4.5.

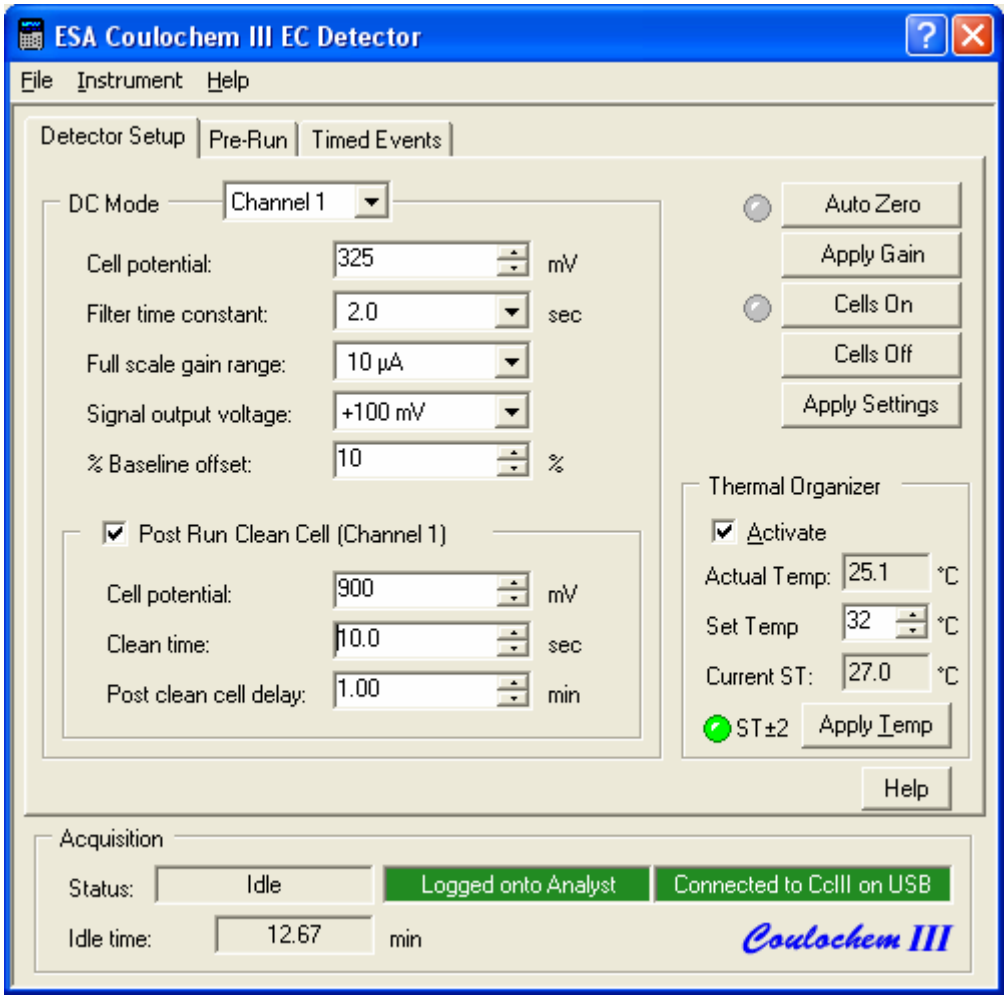


Figure 4.5. Detector Tab Parameters for DC Mode

Note that when channel 2 and/or the guard channel are enabled, the same fields are used to enter parameters for channel 1, channel 2 and the guard cell. Use the drop list control to indicate which channel's parameters you wish to view or modify.

Channel Drop List. Select either Channel 1, Channel 2 or Guard. Selections allowed depend on the system configuration parameters, so, for example, Channel 2 will not appear in the list if the checkbox *Enable channel 2* was not checked in the ESA Coulochem III Detector Configuration dialog (see section 3).

WARNING: Remember to set potentials, filter constants, etc. for all channels in the configuration. Forgetting to set a channel's parameters may lead to unexpected experimental results.

Cell potential. Select the cell potential for the designated channel, in mV. The "spin buttons" in this field change the potential 10 mV at a time. (The up and down arrows on the keyboard have the same effect.) For more exacting control, enter the required potential from the keyboard. The range is -2000 to +2000 mV. The choice of potentials depends on the oxidation-reduction properties of the compounds being investigated. Typically, the choice of potentials is made after running a series of stepped potentials over multiple injections of the compounds of interest.

Filter time constant. Values of 0.2, 0.5, 1.0, 2.0, 5.0 and 10.0 sec are allowed. The value of this parameter is only relevant when monitoring the EC signal using the ADC. A small filter constant provides the least smoothing of data. Optimizing this parameter is primarily relevant when using the EC cell for highly sensitive detection. In that case, select the lowest filter constant needed to improve the signal-to-noise ratio. Default is 5.0 sec.

Full scale gain range. The full scale gain range runs from 10 pA to 1 mA in steps of 1, 2, 5, 10, 20, 50, 100, 200 and 500. The value of this parameter is primarily relevant when monitoring the EC signal using the ADC. Set this value above the highest current expected for the analyte of interest over the course of the experiment. Default is 100 µA.

Signal output voltage. This is the maximum analog signal the detector will output to a strip chart recorder or ADC unit. Select from values of -1 V, -100 mV, +100 mV and +1 V. Default is +1 V.

% Baseline offset. This is the ratio by which a flat signal baseline is offset on a chart recorder or ADC unit, expressed as a % of full scale. Offset values from -50% to +50% are allowed. Default is 0 %.

Post Run Clean Cell. Check this box if the selected channel is to be used in post-run cleaning. A clean cell period is used to help desorb products that may have a high affinity for the electrodes, and is typically used with potentials that are higher than those normally used in detection. Clean cell is normally used only when indicated, typically when the signal for a certain compound begins to decline from its normally observed value after repetitive injections.

Cell potential (clean cell). Select the post-run potential for cleaning the selected channel (electrode). The range for clean cell potentials is -2000 mV to +2000 mV. When using a clean cell cycle, use a potential that is higher than that normally used for the compound(s) being detected.

Clean time. Clean times may be selected from a minimum of 1 sec to a maximum of 120 sec. Typical clean cell times are 30 – 60 sec.

Post clean cell delay. This is the amount of time given to allow the detector to stabilize after the clean cell period. Delay times may be selected in the range 0.1 to 10.0 min. Typical clean cell delay time is 0.5 min. or less when using the cell only as a reactor. A longer delay, typically 2 minutes, is required when using EC for sensitive detection and depends largely on the gain range setting.

4.4.1.2 Pulse Mode

Pulse mode method parameters are illustrated in Figure 4.6. Pulse mode is typically used for EC detection of specific analyte classes, such as carbohydrates, that are not readily measured using DC techniques. In this case the parameter settings are typically based on literature values (e.g., ESA Biosciences' Application Notes). This mode however provides a great deal of flexibility to experiment with dynamic waveforms to effect reactions not readily observed with DC for MS study.

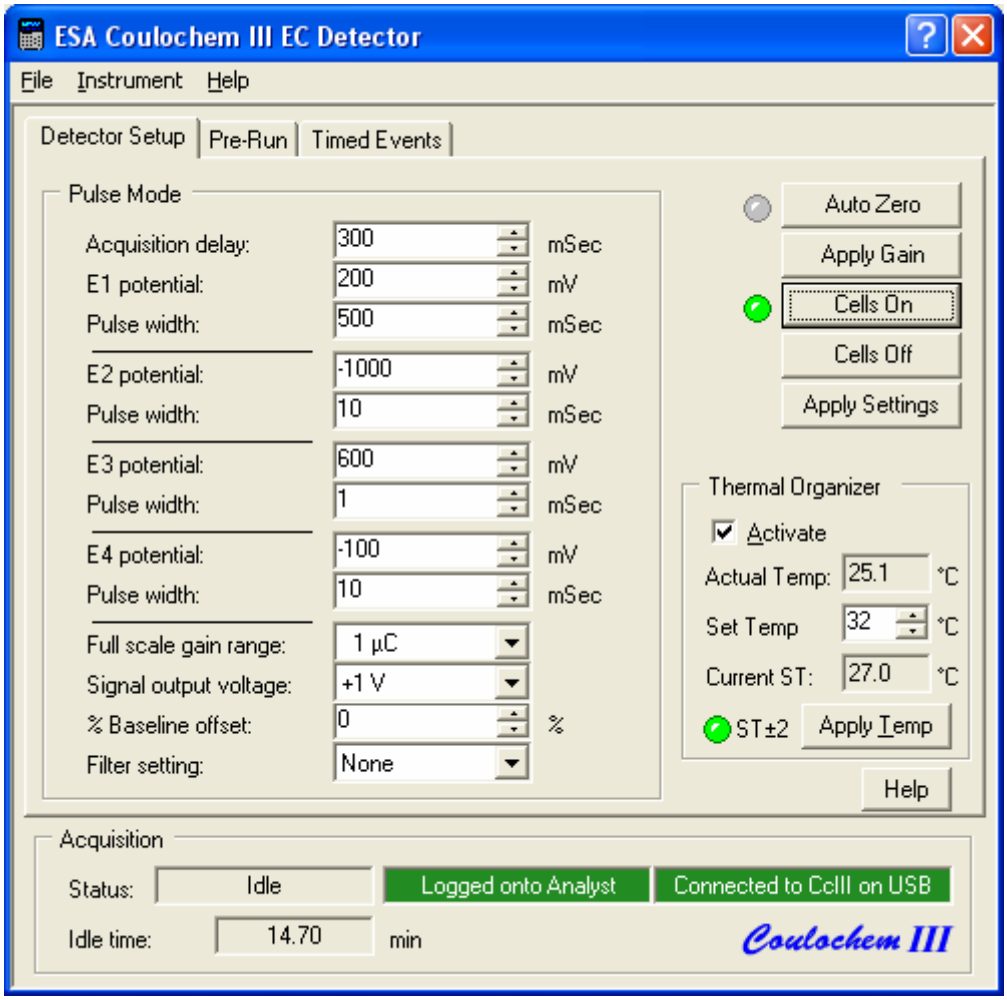


Figure 4.6. Detector Tab Parameters for Pulse Mode

Acquisition Delay. Select a value from 50 to 495 msec (default 300). The value of this parameter is primarily relevant when monitoring the EC signal using the ADC.

E1 – E4 Potentials. Values from -2000 to +2000 mV are allowed. Default values for potentials E1 thru E4 are:

E1:	200	mV
E2:	-1000	mV
E3:	600	mV
E4:	-100	mV

E1 – E4 Pulse Widths. The pulse width for E1 must be at least 5 msec greater than the acquisition delay. Maximum value for all pulse widths is 1000 msec. Minimum and default values are:

E1:	AD+5	msec minimum	500	msec default
E2:	4	msec minimum	10	msec default
E3:	0	msec minimum	1	msec default
E4:	0	msec minimum	10	msec default

Full scale gain range. The full scale gain range runs from 10 pC to 10 mC in steps of 1, 2, 5, 10, 20, 50, 100, 200 and 500. Set this value above the highest signal expected over the course of the experiment.

Signal output voltage. Select from values of -1 V, -100 mV, +100 mV and +1 V. Default is +1 V.

% Baseline offset. Offset values from -50% to +50% are allowed. Default is 0 %.

Filter setting. Select None, Low, Med or High. Use the lowest filter needed to reduce output noise. Default is None.

4.4.2 Pre-Run Tab

Just prior to actual data collection, Analyst provides a "pre-run" period during which any instrument attached to the active profile may delay the start of data acquisition. The Coulochem III driver (see Figure 4.7) provides the user with three options for the pre-run interval. These include a simple pre-equilibration period of up to 2 hrs, autozero prior to run, and waiting to reach the thermal organizer setpoint temperature:

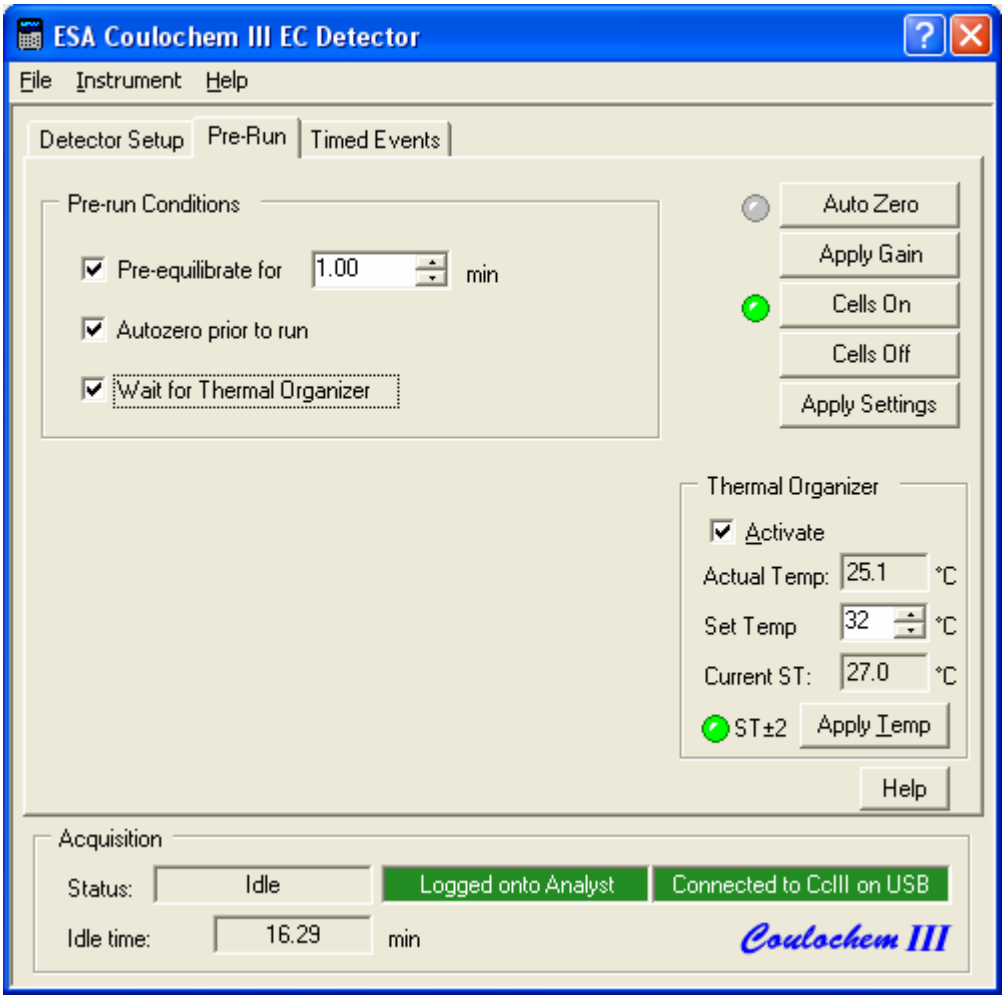


Figure 4.7. Pre-Run Tab Parameters (DC and Pulse Modes).

Pre-equilibrate for *n* minutes. Checking this option and setting a non-zero equilibration time tells the Analyst program to delay data acquisition by at least the period indicated. (Other options may extend it.) This allows the Coulochem III detector to stabilize any new cell potentials between runs. A pre-equilibration period of a few minutes is normally used when transitioning from high to low (or low to high) potentials between samples (and methods). Larger differences in potentials would indicate longer stabilization times.

Autozero prior to run. If this option is checked, the Coulochem III detector will perform an autozero at the end of the pre-run interval. The pre-run interval will automatically be extended as necessary in order to ensure that at least 15 seconds expire for proper detector stabilization after the autozero.

Wait for Thermal Organizer. Checking this option ensures that the pre-run period will not end until the thermal organizer temperature has reached within 2° C of its setpoint. If **Autozero prior to run** is also checked, the autozero is performed only after the thermal organizer has reached its setpoint.

Pre-run options are available for both DC and Pulse mode operation of the Coulochem III detector.

4.4.3 Timed Events Tab

The Timed Events tab (Figure 4.8) lets the user specify various actions to be performed during data acquisition. These actions include:

- adjusting the gain range
- adjusting the cell potentials (DC mode only)
- performing an autozero
- turning cells off
- placing an event marker in the data

Events are entered by selection from a drop list. Channel value is selected by drop list, but may be grayed out if not applicable to the line's event. Value entries may be made by drop list or manually, depending on the event, and may be grayed out if not applicable to the line's event.

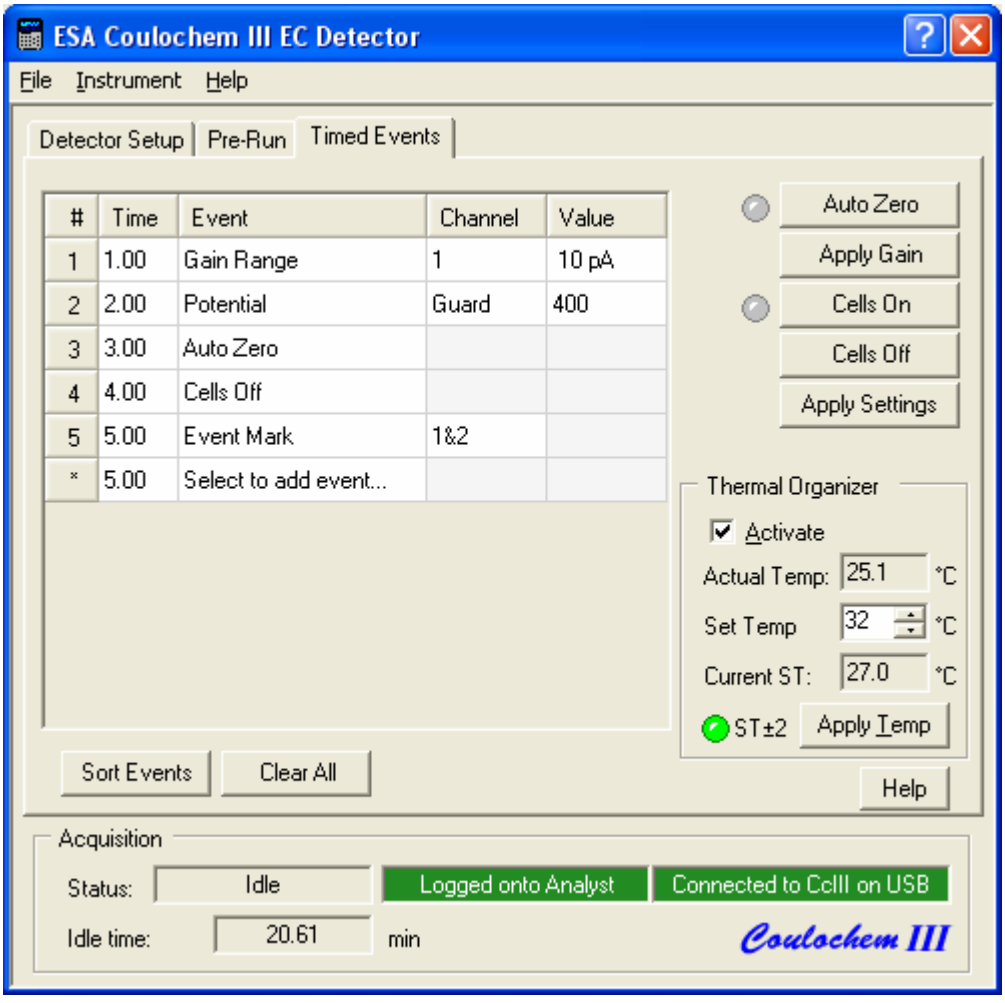


Figure 4.8. The Timed Events Tab with Event Examples

The total duration of Coulochem III data acquisition is determined by the greater of the time of the last event (after sorting) and the MS acquisition time.

Each timed event consists of a time point and an event type. Some events may also include an event channel as well as an event value.

Timed events are tabulated in the Timed Events grid. The default grid has no events. To add an event, simply click in the cell that reads "Select to add event...", then choose an event from the drop list. Click in either adjacent cell to "activate" the event. Edit the event's time, in minutes, and, if a cell has either a channel option or an associated value, select or edit the appropriate channel or value cell. Cells with no expected entries are displayed in light gray.

Depending on personal preference, timed events can be entered using primarily the mouse or the keyboard. When primarily using the keyboard, each time the Tab key is pressed the focus moves to the next cell in the row. Pressing the Tab key at the end of a row advances the focus to the Time column of the next row. (Shift Tab reverses the flow.) When a cell has the focus, its contents can be edited by using the up and down arrow for drop lists (Event, Channel and Gain Range Value) or by editing directly (Time and Potential Value).

Time. Specifies the time of the event in minutes from the beginning of EC data acquisition.

Event. Specifies the event to occur at the specified time.

Channel. Specifies the channel or channels on which the event is to apply. The channel is irrelevant in Pulse mode. In DC mode, the channel can be specified for the Gain Range, Potential and Event Mark events only.

Value. Specifies the value for the event. This applies only to the Gain Range and Potential (DC mode only) events.

The following events can be designated:

Gain Range. This event changes the gain range to a new value, selected from the drop list in the Value field, at the specified time into the run.

Auto Zero. This event implements an autozero at the specified time. An autozero event must not occur at the end of the timed events. Furthermore, the next event following the autozero event must not occur before 15 sec (0.25 min) have elapsed since the autozero. These conditions are checked prior to attaching the method properties to an Analyst file; a warning is provided if the conditions are not met.

Cells Off. This event turns the cells off at the specified time. Once turned off, cells cannot be turned back on by another timed event. (There is no Cells On

event.) However, cells can be turned back on manually by clicking the Cells On button on the driver window.

Event Mark. This event sets a marker spike in the raw analog data at the specified time into the run. In DC mode, the event can indicate which channel or channels are to receive the marker. Marker height depends on the value provided in the Coulochem III Configuration dialog (Section 3.4).

Potential. This DC-mode-only event changes the cell potential to a new value given by the Value field, in mV, on the channel selected from the list box in the Channel field, at the specified time into the run.

Click the **Sort Events** button to sort the events by time.

Click the **Clear All** button to clear all events from the Timed Events grid.

4.4.4 Thermal Organizer Controls

If the thermal organizer option is included in the Coulochem III system (and this has been indicated in the Coulochem III Configuration dialog - Section 3.4), controls for the thermal organizer will appear in the driver window (Figure 4.9).

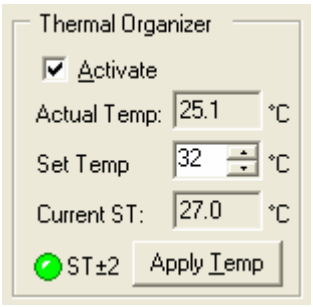


Figure 4.9. The Thermal Organizer Group Controls

To activate the thermal organizer, check the Activate checkbox. When active, the group of controls in the Thermal Organizer group box indicate actual and setpoint temperatures in Celsius. The display is updated about every second. The following describes each of the controls in the Thermal Organizer group:

Activate. Check this checkbox to activate the thermal organizer. Uncheck to disengage the organizer. When disengaged, the organizer has no effect on temperature control.

Actual Temp. This is the actual temperature in the thermal organizer chamber, updated every second.

Current ST. This is the current active setpoint temperature stored within the Coulochem III detector.

Set Temp. This edit field allows the user to change the Coulochem III's stored setpoint temperature. The **Apply Temp** button (see below) must be clicked in order for the new Set Temp to be applied to the Coulochem III.

Apply Temp button. Click this button to change the Coulochem III's stored setpoint temperature to the value provided in the Set Temp field.

ST±2 Indicator lamp. This indicator turns green whenever the thermal organizer's actual temperature is within 2° C of its setpoint temperature.

4.4.5 Attaching the Method to an Analyst File

The Coulochem III constituents of the data acquisition method consist of all of the parameters found on the Detector Setup tab, Pre-Run tab, Timed Events tab and Thermal Organizer control group (if active). In order for these parameters to become part of an Analyst method, they must be attached to an existing Analyst acquisition method (*.dam) file.

To attach the Coulochem III method parameters, select **File > Attach Method to Analyst File...** and select the Analyst acquisition file from among the *.dam files listed. Click **Save** to attach and save the modified method. Note that an error message will appear if you enter a new file name and click the **Save** button. The Coulochem III method data must be saved to an existing Analyst acquisition method.

Remember that without this step, the Coulochem III method data will not be known by Analyst and will not be available to it.

4.4.6 Viewing the Coulochem III Method from within Analyst

Once the Coulochem III method data has been attached to an Analyst acquisition method file, its parameters can be viewed from within Analyst. To view the method, follow these steps:

1. Select and open the *.dam file in Analyst by selecting **File > Open** from the Analyst menu, then selecting **Acquisition Method (*.dam)** from the drop list. Select the file from the file list.

2. Click on **Coulochem III Detector** in the left panel of the Acquisition method display. The Software Application Properties tab will display all of the Coulochem III configuration data, pre-run conditions, thermal organizer

settings, mode parameters (DC or Pulse) and timed events for the method, as illustrated in Figure 4.10.

To print the Coulochem III method data, right click anywhere in the tabulated data and select **Print** from the context menu.

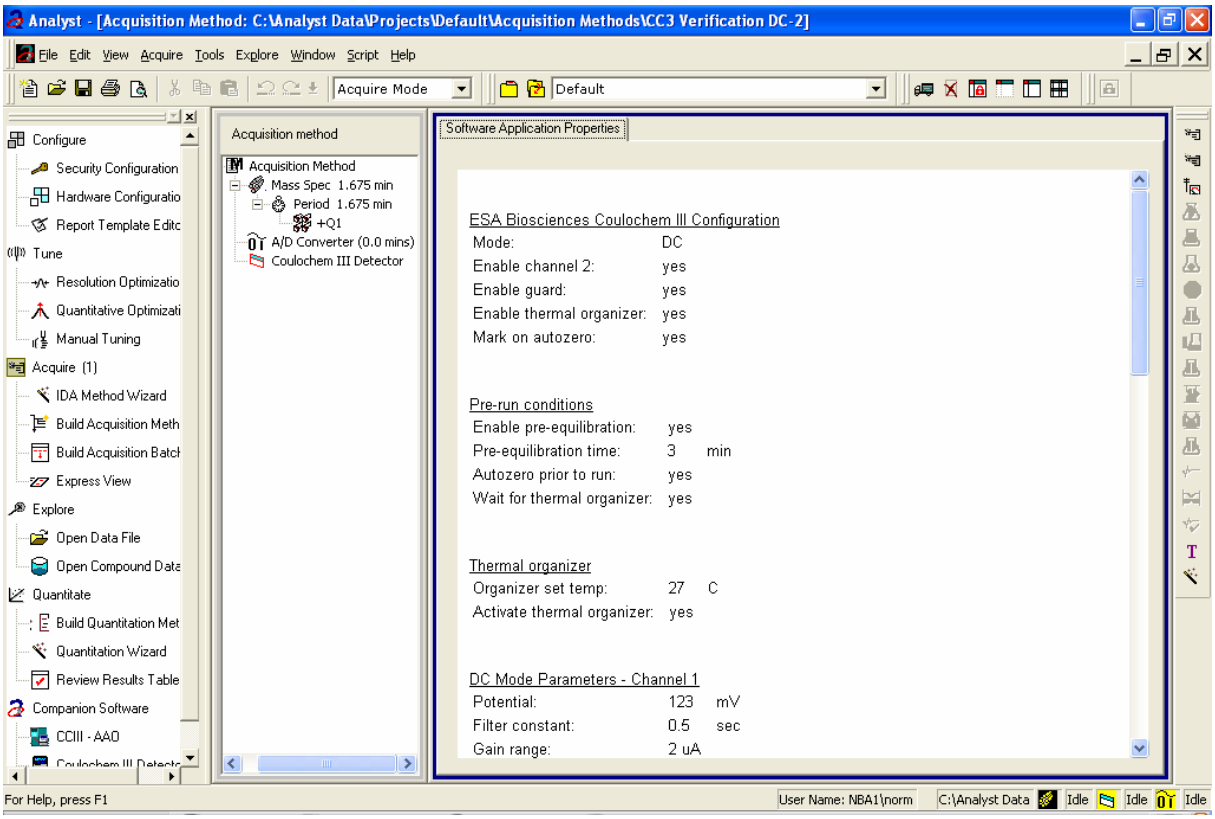


Figure 4.10. Analyst Program Showing Coulochem III Method Properties

4.5 Modifying an Attached Coulochem III Method

Once Coulochem III method data has been attached to an Analyst acquisition, it is possible to modify the file data at any time. However method data that is in use in an actively acquiring method cannot be modified.

To modify the Coulochem III method data that is attached to an Analyst file, select **File > Read Method from Analyst File** from the Coulochem III driver menu, select the *.dam file to be read, and click **Open**. This loads the method data into the driver window. The data can then be modified as required. To save and reattach the data to an Analyst file, select **File > Attach Method to Analyst File...** and select the Analyst acquisition file from among the *.dam files listed. (It does not have to be the same file that was previously read.) Click **Save** to attach and save the modified method data.

4.6 Modifying a Coulochem III Method While Acquiring Data

During active data acquisition the parameters of the actively running Coulochem III method are loaded and displayed in the Analyst driver window and tabs. All fields are disabled in order to prevent modification during a run. However, it is still possible to modify any acquisition method during acquisition; a separate menu item and dialog are available for this purpose.

To modify an existing attached method, select **File > Edit Method from Analyst File** from the driver menu, select the file from the list and click **Open**. The method editing dialog shown in Figure 4.11 will appear.

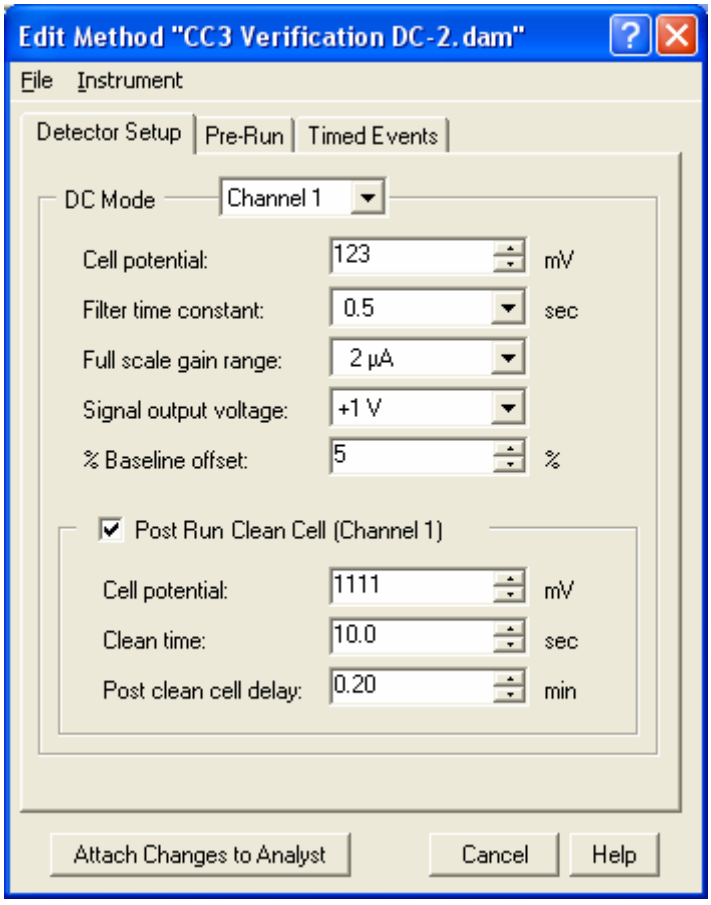


Figure 4.11. Method Editing Dialog.

This dialog has all of the same tabs as the main driver window, and editing is done in exactly the same way as described in the sections above. To view or modify the configuration parameters, select **Coulochem III Configuration** from the dialog's **Instrument** menu. To view or modify the Thermal Organizer parameters, select **Thermal Organizer** from the dialog's **Instrument** menu. In either case, a new sub-dialog will appear with all of the same parameters as are found on the main driver interface. Remember to click the **OK** button when closing the sub-dialog if you wish to save any changes made.

To attach the modified method data to the original Analyst file, click the **Attach Changes to Analyst** button at the bottom of the editing dialog, or select **File > Attach Changes to Analyst File** from the dialog's menu.

Click the **Cancel** button if you decide not to change the original Analyst file.

WARNING. It is possible to modify the actively running acquisition method using this dialog. However, when a sample is submitted to the sample queue, the then-current values of the method parameters are stored in the queue's sample parameters. Modifying a method after samples have been added to the queue will not change the method parameters in the existing queue samples from their original values, but samples added to the queue after the method change will show the changes. Further, once the acquisition method parameters have been loaded into the Coulochem III driver, just prior to the run for each sample, they remain fixed throughout the run.

Keep this in mind when modifying Coulochem III method parameters. The modifications will not affect samples already submitted to the queue, but they will affect new samples added to the queue after the modification.

5. Basic Coulochem III Driver Functions

5.1 Overview

The Coulochem III driver provides some basic detector functionality even when not actively involved in data acquisition, such as the ability to apply settings to the Coulochem III detector, perform an autozero, and turn cells on and off. This section examines these independent functions, and begins with a discussion of the behavior of the driver during startup and shutdown as it connects or disconnects with the Coulochem III detector.

5.2 Startup and Shutdown Sequences

5.2.1 Startup Sequence

Immediately after confirming the connections to both Analyst and the Coulochem III detector, the Coulochem III driver recalls the last configuration it was in (DC vs. Pulse mode, Enable channel 2, Enable guard cell, etc.) prior to shutdown, and attempts to reestablish the same configuration. It then disables most of the keys on the Coulochem III keypad, sends default method parameters to the Coulochem III for the recalled mode and starts the Coulochem III in DC or Pulse mode.

After completing this sequence, the Coulochem III detector display panel will show the current default mode settings.

This startup behavior occurs whether the Coulochem III driver is initiated from the icon on the desktop, from the Companion Software items in Analyst or as the result of activating an Analyst hardware profile.

5.2.2 Shutdown Sequence

After the user attempts to close the Coulochem III driver (by **File > Exit** or other standard means), the driver stops the Coulochem III detector, unlocks the keypad and restores the detector display to the main menu. It then stores the current configuration data before finally closing.

5.3 Direct Coulochem III Detector Control

The main display surface of the Coulochem III driver provides a set of 5 buttons for directly controlling the Coulochem III detector. These buttons are shown in Figure 5.1 below.

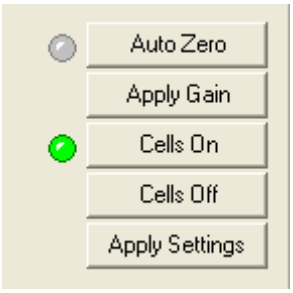


Figure 5.1. The Driver's Detector Control Buttons and Indicators

Auto Zero. Click this button to autozero the detector. "Autozeroing" adjusts the gain to produce a zero signal. In a 2-channel setup in DC mode, the autozero is applied across both channels. The **Auto Zero** button is disabled during the pre-run, acquisition and post-run (clean cell) phases of a run.

In DC mode, post-autozero stabilization can last up to 15 seconds (or more), while in Pulse mode it is nearly instantaneous. During autozero the indicator next to the **Auto Zero** button lights green.

Apply Gain. Click this button in DC mode to apply the current full scale gain range to each enabled channel. The **Apply Gain** button is disabled in Pulse mode. The button is also disabled during the pre-run, acquisition and post-run (clean cell) phases of a run.

Cells On/Off. Click either of these buttons to turn the cells on or off. When on, the indicator next to the **Cells On** button lights green. These buttons always remain enabled, even during a run.

Apply Settings. Clicking this button sends all of the current settings (DC or pulse) to the Coulochem III detector and resets the detector to activate the new values. The **Apply Settings** button is disabled during the pre-run, acquisition and post-run (clean cell) phases of a run.

5.4 Thermal Organizer Control

If the system is equipped with a thermal organizer, and the organizer is enabled, the **Apply Temp** button (see Figure 4.10) is used to set the setpoint temperature (from the Set Temp field) in the Coulochem III. The **Apply Temp** button is disabled during the pre-run, acquisition and post-run (clean cell) phases of a run.

The ST±2 indicator turns green whenever the thermal organizer's actual temperature is within 2º C of its setpoint temperature.

For more on the thermal organizer, see Section 4.4.4.

6. Acquiring Data with the Coulochem III Driver

6.1 Overview

The basic steps in running samples with the Coulochem III driver are:

1. Start Analyst

2. Activate a hardware profile. *See Section 4 for more on hardware profiles involving the Coulochem III detector.*

   Note: Once a profile has been activated it is normally not necessary to deactivate the profile when closing the Analyst program (unless other components require deactivation; the Coulochem III driver does not). If Analyst is closed without deactivating the active profile, restarting Analyst will automatically restart the Coulochem III driver.

3. Build acquisition methods as appropriate for the samples. The same method may be used for all samples, or different methods may be used for different samples.

   Remember to attach Coulochem III parameters to the method. See Section 4 for more on building acquisition methods.

4. Build an acquisition batch.

5. Submit batch samples to the batch queue.

6. Run the samples in the queue.

Figure 6.1 illustrates a simple 5-sample batch using method "DC 2ch with Guard".

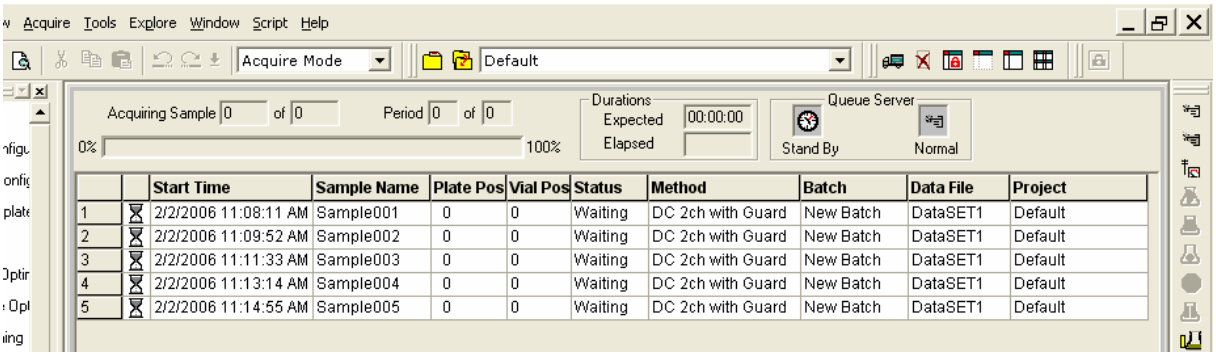

Acquire Tools Explore Window Script Help
Acquire Mode
Default
Acquiring Sample 0 of 0
Period 0 of 0
0%
100%
Durations
Expected 00:00:00
Elapsed
Queue Server
Stand By
Normal
Start Time | Sample Name | Plate Pos | Vial Pos | Status | Method | Batch | Data File | Project
1 | 2/2/2006 11:08:11 AM | Sample001 | 0 | 0 | Waiting | DC 2ch with Guard | New Batch | DataSET1 | Default
2 | 2/2/2006 11:09:52 AM | Sample002 | 0 | 0 | Waiting | DC 2ch with Guard | New Batch | DataSET1 | Default
3 | 2/2/2006 11:11:33 AM | Sample003 | 0 | 0 | Waiting | DC 2ch with Guard | New Batch | DataSET1 | Default
4 | 2/2/2006 11:13:14 AM | Sample004 | 0 | 0 | Waiting | DC 2ch with Guard | New Batch | DataSET1 | Default
5 | 2/2/2006 11:14:55 AM | Sample005 | 0 | 0 | Waiting | DC 2ch with Guard | New Batch | DataSET1 | Default

Figure 6.1. A Simple Batch with 5 Samples

Instructions for building acquisition batches and running the queue samples can be found in the Analyst manual and are no different when involving the Coulochem III detector and driver. Refer to the manual for details.

6.2 Viewing the Progress of a Coulochem III Acquisition

As the Analyst program manages the acquisition of each sample in a batch, it dispatches information at various times to each of the instruments or drivers in the active hardware profile, including the Coulochem III driver. Just prior to the start of the pre-run phase, Analyst provides the Coulochem III driver with all of the Coulochem III detector-specific configuration and method data for the sample that is about to be run. Analyst obtains this data from the acquisition method file (and its attached Coulochem III method data). The Coulochem III then updates its interface with these values.

As the run begins its pre-run phase, the Coulochem III driver automatically switches to the Pre-Run tab and begins to display the pre-run time in the Acquisition status region at the bottom of the driver window (Figure 6.2). The Pre-run phase is also designated in yellow in the Status indicator in this region.

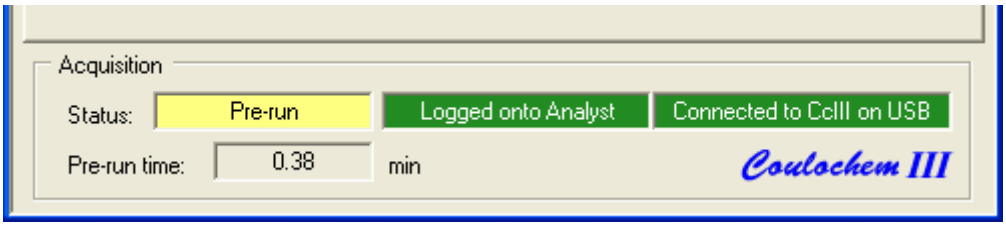


Figure 6.2. Acquisition Status Bar During Pre-Run Phase.

The duration of the pre-run phase depends on the parameters activated and assigned in the Pre-Run tab. After the pre-equilibration time designated in the **Pre-equilibrate for** field, the driver will extend the pre-run phase by as much time as needed for the thermal organizer to reach within 2° C of its setpoint (if activated and checked). If checked, the autozero is performed only after the **Pre-equilibrate for** time has elapsed and the setpoint has been approached. An additional 15 seconds is added post-autozero to ensure adequate stabilization from the autozero event.

The acquisition phase begins immediately after the pre-run phase. The Coulochem III driver automatically switches to the Timed Events tab and begins to display the run time in the Acquisition status region at the bottom of the driver window (Figure 6.3). The acquisition phase is designated in green in the Status indicator.

As the run progresses, and as each timed event is processed in the timed events grid, the last-executed event is highlighted.

Note that during the entire run the Cells On and Cells Off buttons are the only controls that are accessible to the user. All other user input is disabled.

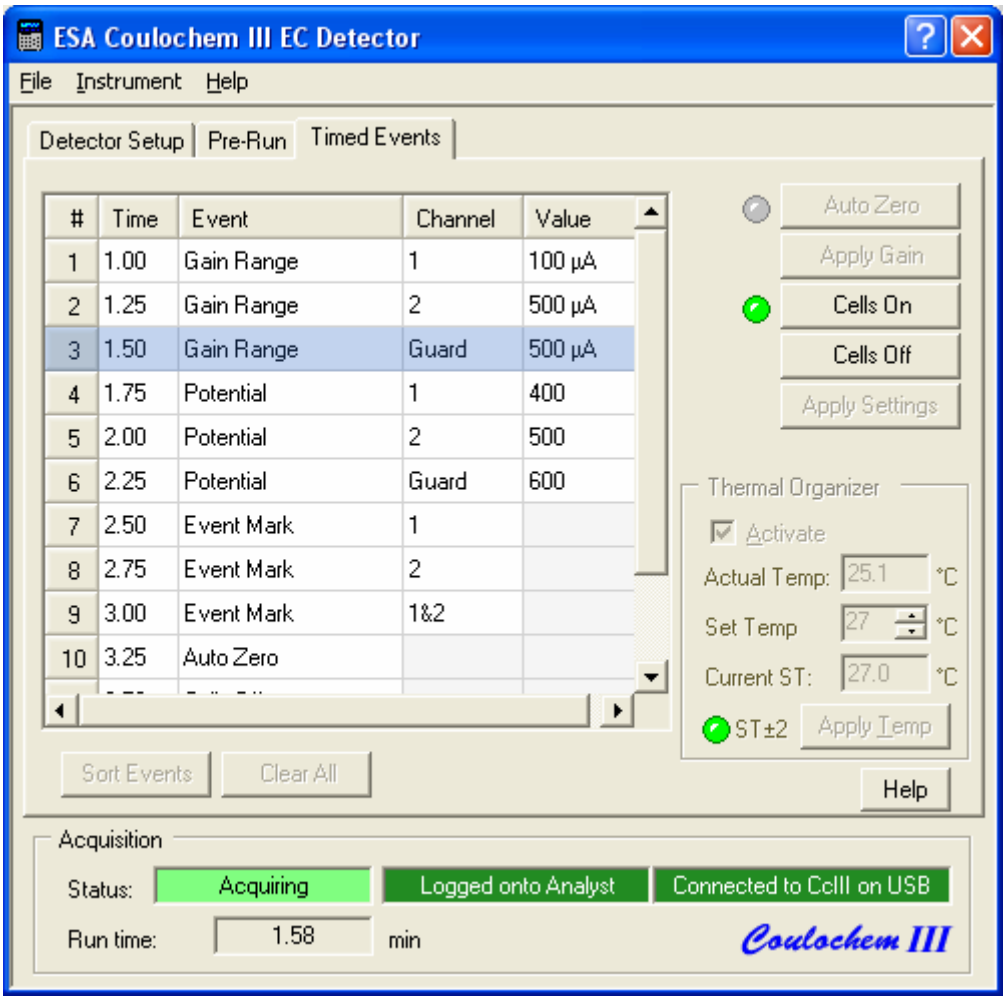


Figure 6.3. Driver Display During the Acquisition Phase of a Run

After the last timed event has been processed, the post-run phase begins. This phase involves cleaning the cells, so the driver switches to the Detector Setup tab, where the clean cell properties are displayed. The phase lasts for as long as the longest combination of Clean time and Post clean cell delay for either channel or the guard cell that is enabled.

During the post-run phase the Coulochem III driver displays the post run time in the Acquisition status region at the bottom of the driver window (Figure 6.4). The post-run phase is designated in blue in the Status indicator.

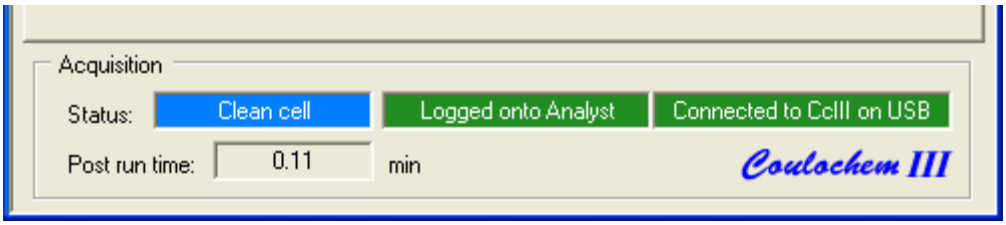


Figure 6.4. Acquisition Status Bar During Post-Run (Clean Cell) Phase.

After all 3 phases of the run have been completed, the driver reverts to the idle state and displays idle time in the Status indicator, in preparation for the next sample. The 3-phase acquisition repeats for each remaining sample in the Analyst queue, until all samples have been run.

6.3 Aborting a Batch from the Driver Program

A batch can be aborted from within the Coulochem III driver during any phase of the acquisition. To stop the batch, select **Instrument > Abort Batch** from the driver menu. Aborting a batch causes the driver to shift immediately to the post-run (clean cell) phase of the currently executing run. The driver also sends a signal to Analyst to stop any further execution of the batch.

6.4 Viewing the Coulochem III Electrochemical Data Profile and Method Properties

During the acquisition phase of a run, the analog electrochemical data signal is sent to the A/D Converter box, converted to a digital stream of data and fed into the Analyst program for storage inside an Analyst

To view the electrochemical profile(s), select **File > Open Data File** from the Analyst main menu. Then select the data file from the files listed, and then the sample name, and click **OK**. Analyst opens the file and displays the total ion chromatogram (TIC) data by default. To view the electrochemical profile(s), right-click in the TIC plot and select **Show ADC Data** from the context menu. Select **A/D Converter – Channel 1** and click **OK**, then repeat to display channel 2 if using 2 channels. Figure 6.5 shows an example of electrochemical data profiles for 2 channels.

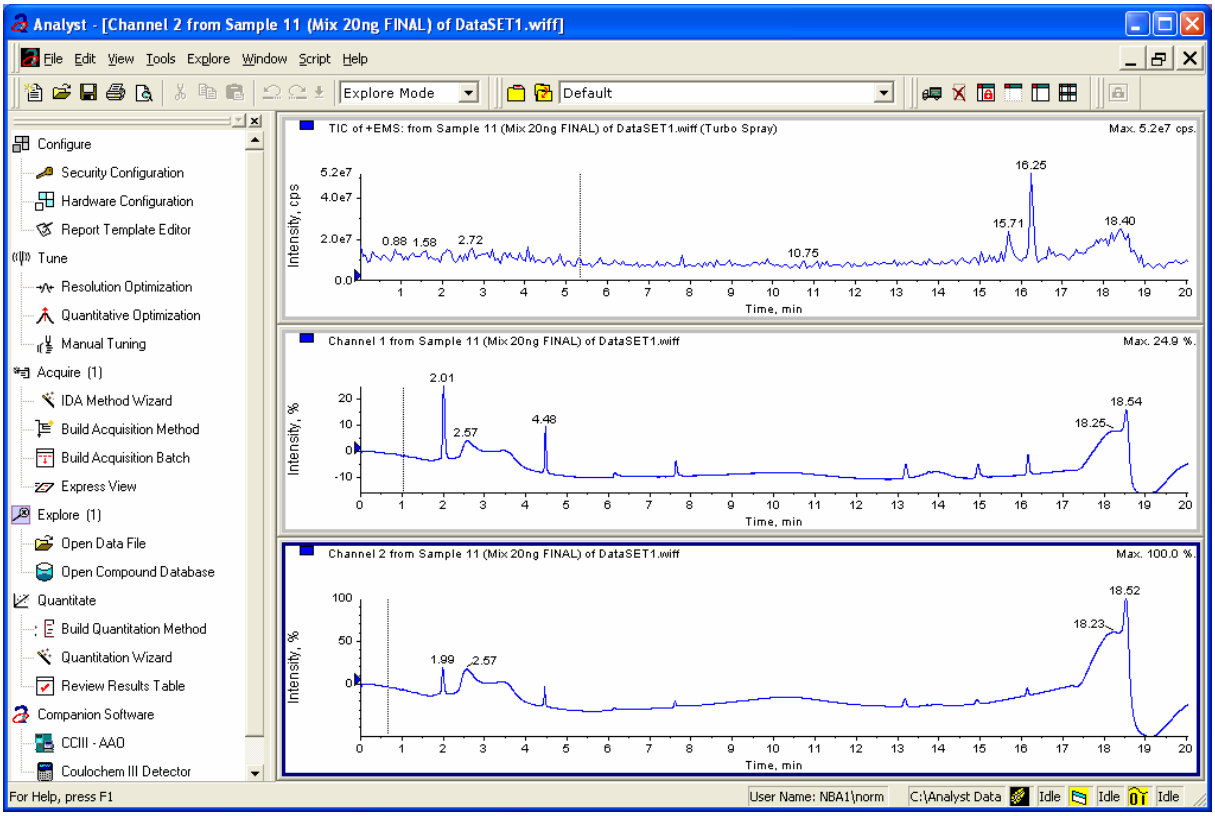


Figure 6.5. Example Electrochemical Data Profiles in Analyst (Bottom 2 Panels)

To view the method properties associated with the file, highlight any plot and select **Explore > Show > Show File Information** (or press Ctrl-I on the keyboard). Analyst displays a considerable amount of property data for the file, including a section for Software Application Properties. This section displays the Coulochem III method properties, as shown in Figure 6.6.

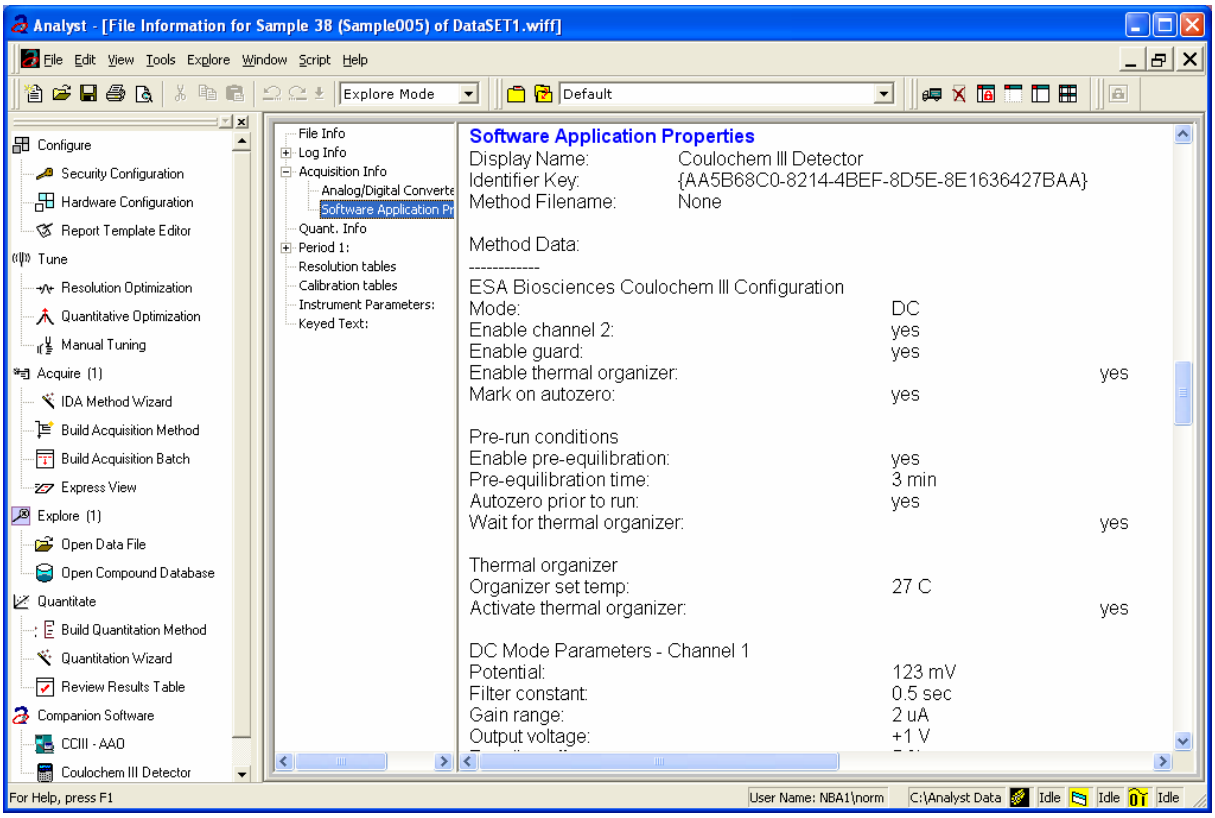


Figure 6.6. Coulochem III Method Properties Displayed in a Data File's Software Application Properties

7. Application Examples

7.1 Overview

This chapter provides examples of the use of the Coulochem III detector to control electrochemical (EC) cells with fixed (DC) or dynamic (pulsed) potential waveforms. Cells can be used as reactors in series upstream of the MS ion source and/or as detectors in parallel (via flow split) with MS. Common applications are for on-line derivatization (e.g., to assist MS ionization, for mass tagging and protein cleavage) and to study the susceptibility of compounds to oxidation (or reduction), the nature of products and short-lived intermediates, and the associated reaction pathways. These applications are of interest for generating and characterizing products that often correspond to biological metabolites, degradants or reactive species of interest. The use of EC in parallel with MS provides complementary, orthogonal, detection with high sensitivity and selectivity for redox active species. Typical applications are DC detection of antioxidants and markers of oxidative stress and pulsed detection of carbohydrates.

Each example tabulates the conditions for the liquid chromatography (LC) setup, Coulochem III (EC) setup and mass spectrometer (MS) setup.

7.2 Assisted Ionization – DC Mode

Many neutral or non-polar compounds do not ionize very readily. However, oxidative derivatives of electrochemically active compounds may be more readily ionizable. In this example, the Coulochem III is used to control an EC reactor cell placed in series, upstream, with an electrospray ion (ESI) source. The EC cell is used to convert solution-phase neutral species of butylated hydroxyanisole (BHA) to those that are more readily ionized for ESI-MS analysis.

LC Conditions

- Mobile phase: 50% MeOH, 20 mM NH_4Acetate
- Flow rate: 0.1 ml/min
- Injection: 10 µl BHA, 20 µg/ml

EC Conditions

- ESA Model 5021A Conditioning (Reactor) Cell
- Ground Decoupling Kit (ESA P/N 70-5971) connected between EC cell and MS.
- Cell potential: 600 mV
- Filter time constant: 0.5 sec
- Full scale gain range: 10 µA
- Signal output voltage: +1V
- Baseline offset: 0 %
- Pre-equilibration: 2 min, autozero prior to run

MS Setup

- Electrospray: positive ion mode
- Q1 Scan 100 to 500 *m/z*

Results

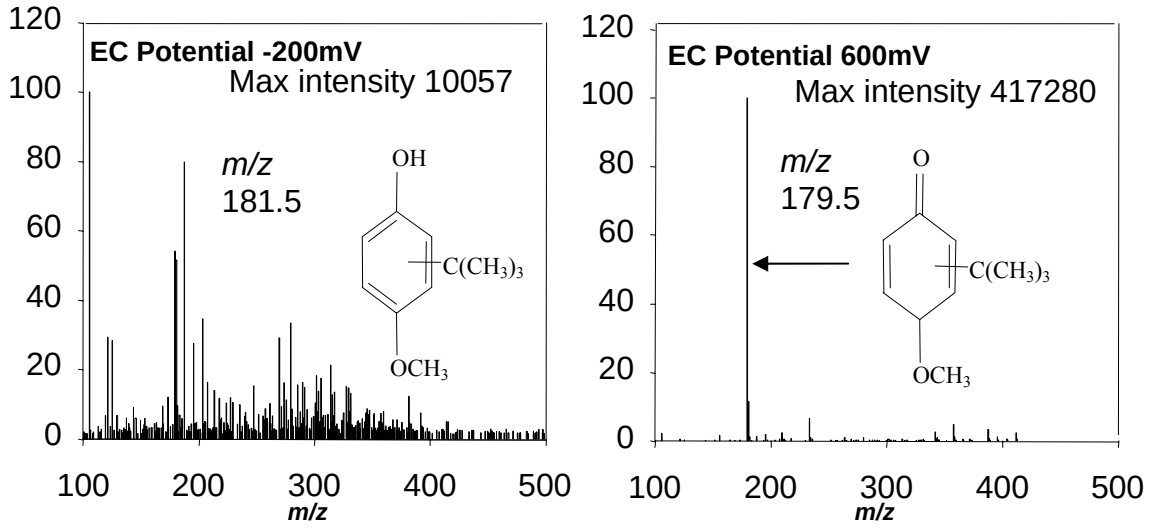


Figure 7.1. Results of Assisted Ionization Run with BHA

The left panel in Figure 7.1 above shows an injection of 200ng of BHA with the EC cell at -200mV, a potential at which no EC reaction is expected. The spectrum is very low intensity with little evidence of protonated BHA (*m/z* 181.5). The right panel shows a similar injection with the EC cell potential at 600mV. There is an intense peak at *m/z* 179.5 representing an oxidized species derived from BHA. This is a simple demonstration of the ability of EC to convert a solution-phase neutral species to a more readily ionized species. The ability to automate control of the EC cell potential and change potential within a run (via timeline) and/or between runs (via different methods in an Analyst batch) provides a significant amount of experimental flexibility.

7.3 Metabolite Identification – DC Mode

Typical applications of serial EC-MS involve on-line generation and analysis of EC products using LC-MS conditions that are typical of metabolic studies (e.g., in vitro microsomal analysis). Serial EC/LC-MS can thus be used with 'neat' parent compound solutions for preliminary optimization of LC and MS conditions for subsequent metabolite analysis in biological samples. By using identical conditions, EC data may then be used as input to automated metabolite identification software (i.e., Analyst® Metabolite ID) to aid in finding metabolites present in more complex biological matrices. Furthermore, when the data from an EC-generated product corresponds to that of a biological metabolite, the EC technique may then be viewed as a selective and rapid synthetic route to small quantities of this metabolite. This can then be used to facilitate structural elucidation or, more generally, to produce additional chemical entities. The following example shows the utility of the Coulochem III Analyst Driver for automated analysis of tamoxifen with an EC reactor cell controlled at different oxidative potentials for each injection.

LC Conditions

- Column: Shiseido C18 MG 3µm 4.6 mm ID x 75 mm
- Mobile phase gradient:
  - A: 1% ACN, 20 mM NH_4Acetate
  - B: 80% ACN, 20 mM NH_4Acetate

Time	%B	Flow (ml/min)
0.00	20	1
10.00	100	1
12.00	100	1
12.01	20	1
15.00	20	1

- Flow rate: 1 ml/min
- Injection: 10 µl Tamoxifen, 20 µg/ml (200 ng)
- After diode array, flow splitter (ESA P/N 70-6228) adjusted to allow 200 µl into the MS.

EC Conditions

- ESA Model 5021A Conditioning (Reactor) Cell
- Ground Decoupling Kit (ESA P/N 70-5971) connected between EC cell and MS.
- Cell potential: 500 mV, repeat at 1000 mV
- Filter time constant: 0.5 sec
- Full scale gain range: 10 µA
- Signal output voltage: +1V
- Baseline offset: 0 %
- Pre-equilibration: 2 min, autozero prior to run

MS Setup

- Electrospray: positive ion mode
- Enhanced MS Scan 100 to 500 *m/z*

Results

Figure 7.2 shows results for 200 ng Tamoxifen with the EC cell at a potential of 500 mV. Two forms of Tamoxifen are primarily observed, at *m/z* 358.8 (A) and 372.8 (B).

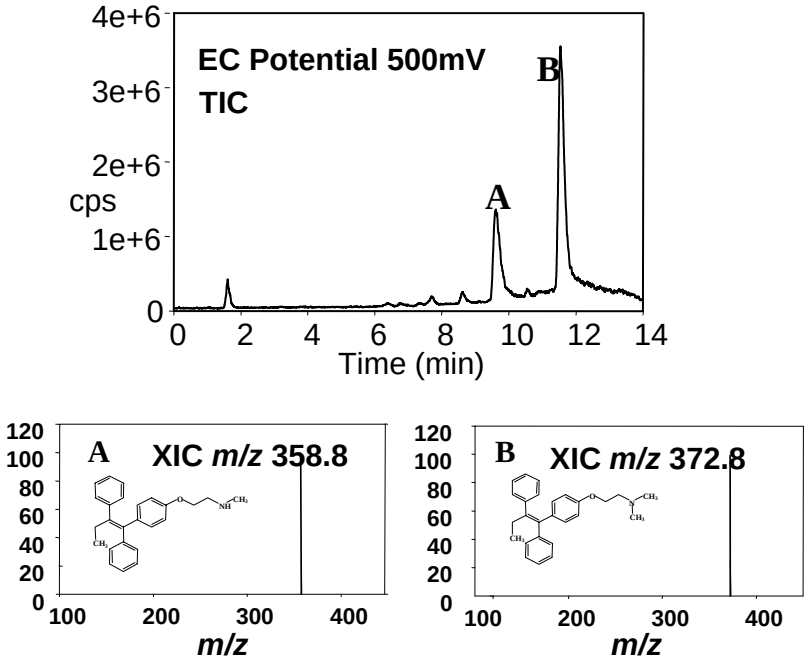


Figure 7.2. Tamoxifen at a Cell Potential of 500 mV

Oxidation at 1000 mV produces 3 *additional* oxidation products (Figure 7.3), with peaks at *m/z* 299.8 (D), 356.8 (A) and 388.8 (B).

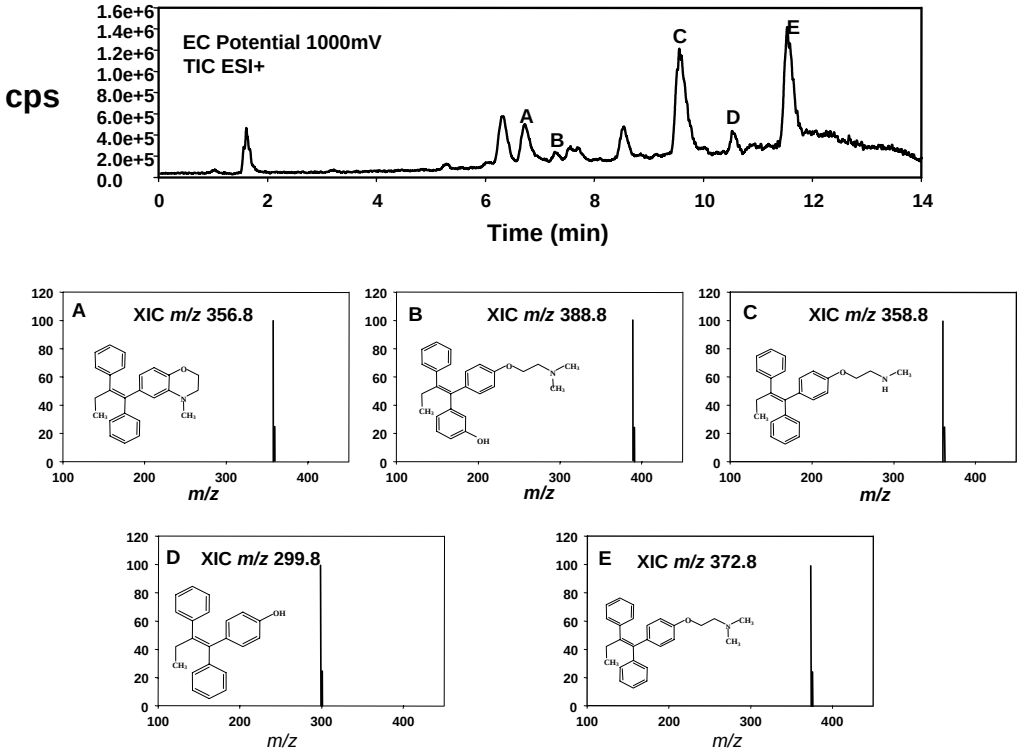


Figure 7.3. Tamoxifen at a Cell Potential of 1000 mV

This example demonstrates that the EC oxidation products for a given compound often coincide with metabolites observed in biological assays. The Coulochem III Analyst driver allowed for automated analysis of tamoxifen using various EC cell potential. The simplicity and speed of on-line EC-LC-MS often provides an effective means of characterizing some of the many unknown metabolites encountered in multivariate profiling studies.

7.4 Carbohydrate Detection – Pulsed Mode

The following example illustrates the use of the Coulochem III Analyst Driver for automated analysis in which a dynamic (pulsed) potential EC waveform is used. In this case the pulsed waveform is used with a gold working electrode EC cell. A typical application is for sensitive EC detection of carbohydrate species in parallel with MS. Serial EC-MS with pulsed waveforms is also of interest in effecting reactions that may not be observed with fixed DC conditions.

LC Conditions

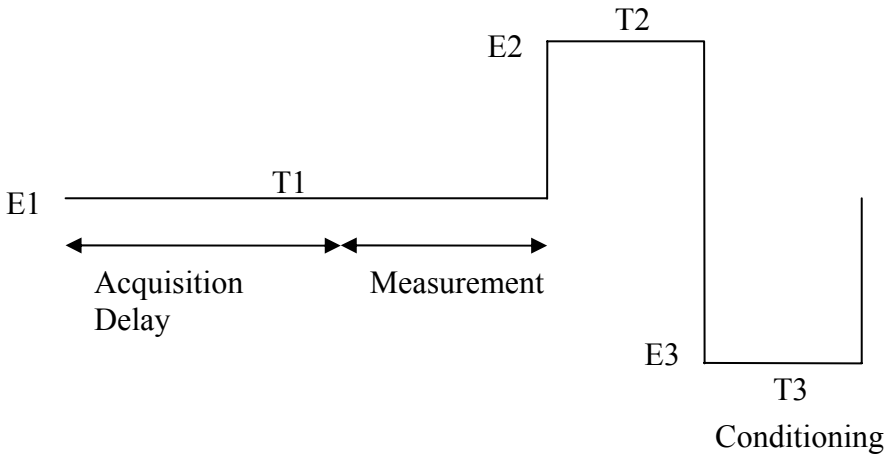
- Column: ESA Carbohydrate RCX-10
- Mobile phase: 100 mM ammonium hydroxide
- Flow rate: 1.5 ml/min

EC Setup

- Analytical cell: ESA 5040 Amperometric cell (gold working electrode)
- Potentials and pulse widths:

	Potential (mV)	Pulse width (msec)
E1	200	500
E2	-1000	10
E3	600	1
E4	-100	10

- Acquisition delay: 300 msec
- Full scale gain range: 1 μC
- Signal output voltage: +1V
- Baseline offset: 0 %
- Filter setting: None
- Cleaning:



Results

Figure 7.4 shows the oxidation profile for a simple mixture of carbohydrates (20 pmoles total).

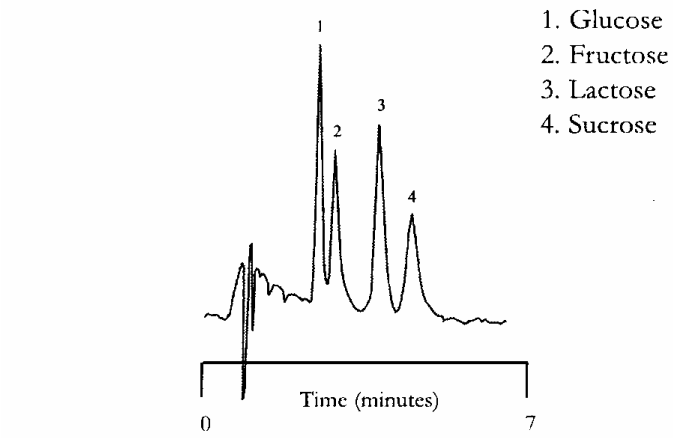


Figure 7.4. EC Detection of a Simple Carbohydrate Mixture

Appendix A: Updating the Coulochem III Firmware

A.1 Overview

The Coulochem III detector is shipped with the latest version of its firmware – the internal software that is responsible for its operation. The firmware version is shown on the Coulochem III front panel display while the detector is running its self-test sequence just after powering on.

Improvements to the software logic may make it advantageous to update the detector's firmware. ESA provides a utility for updating the firmware logic on your Coulochem III. The utility updates only the executable firmware. It ***does not*** update system variables such as the serial number and baud rate, and it ***will not*** affect your stored methods.

This chapter describes how to update the firmware using the Corona-Coulochem Firmware Update Utility CD (ESA P/N 70-6968), which may be obtained from your ESA representative. (The CD uses the same basic utility for updating both the Coulochem III and the Corona CAD instruments.) The steps below detail both the installation and use of the utility.

A.2 Preparation and Installation of the Utility

1. Ensure that the Coulochem III detector is turned off.

2. The Corona-Coulochem Firmware Update Utility cannot operate over the serial port; it must use a USB port. If the Coulochem III has already been connected and operated successfully through the USB port, the required USB driver has already been installed. If not, it may be necessary to install the USB driver. The utility will detect either condition for you.

3. If not already connected through the USB port, a USB cable will be required (standard type A to B). ESA P/N 70-5713 is a 3 meter cable of this type. Connect the small end of the cable to the USB Remote port on the Coulochem III and the wide end to an available USB port on the PC.

4. Close all running software applications. Then insert the Corona-Coulochem Update Utility CD into your CD-ROM drive. The installation program should start automatically (unless CD auto-run is disabled on the PC). If it does not start automatically, click **Start** > **Run**, then type *drive*:\setup (where *drive* is the drive letter of your CD-ROM drive, e.g. D:\setup), then click **OK**.

5. When the installation program starts, click the **Install** button. This will automatically install the Corona-Coulochem Firmware Update Utility program files in the

C:\Program Files\ESA Biosciences folder. It will also ensure that the ESA USB driver is properly installed.

6. When finished, click the **Finish** button.

7. Remove the installation CD and store it in a safe place.

A.3 Updating the Firmware with the Update Utility

1. Ensure that the USB cable is properly connected, as described in the previous section.

2. Turn on the Coulochem III and allow it to complete its power-up tests.

3. Start the update utility by selecting **Start > All Programs > Corona-Coulochem Firmware Update Utility > Corona-Coulochem Firmware Update Utility** from the Windows Start menu. When operating on the Coulochem III, the update utility will appear as in Figure A-1 below.

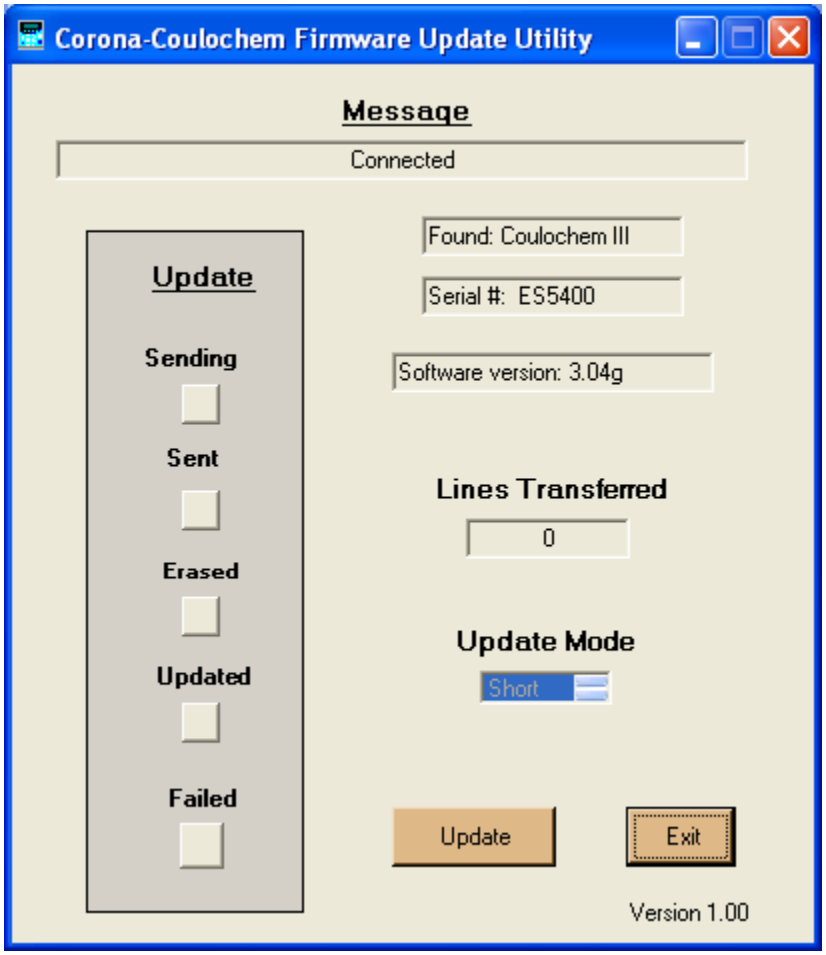


Figure A-1. Firmware Update Utility for Coulochem III

When properly connected through the USB cable, the message "Connected" appears near the top of the utility window, and the utility shows both the serial number and the current firmware (software) version number.

4. Click the **Update** button. When the *Open* dialog appears, navigate to the **Program Files** folder (normally on Drive C), then to the **ESA Biosciences** subfolder and finally to the **Corona-Coulochem Firmware Update Utility** folder (i.e., to the folder *C:\Program Files\ESA Biosciences\Corona-Coulochem Firmware Update Utility*).

5. Select the file coulochem #.##.run, where #.## is the version number of the new firmware update (for example, "coulochem 3.07.run"). If more than one such file is found, select the one with the highest (latest) version number. Then click **Open**.

6. The update utility will replace all of the existing firmware with the version on the update CD. The process should take no more than a few minutes. When finished, the message "Download complete Coulochem III rebooting" will appear near the top of the window and the Coulochem III will reboot automatically.

7. Click **Exit** to close the update utility.

Appendix B: Parameter Ranges and Default Values

B.1 Configuration and Method Parameter Ranges and Defaults

Table B1 below lists all configuration and method parameters, along with each parameter's range, units and default value.

Table B1. Parameter Ranges and Default Values

Parameter	Range	Units	Default
System Configuration			
Mode	DC or Pulse		DC
Communications port	COM1...COM4, USB		COM1
Enable channel 2	yes or no		no
Enable guard channel	yes or no		no
Enable thermal organizer	yes or no		no
Enable event marker on AZ	yes or no		no
Event marker height	-20 to +20	%	5 %
Lock keypad	yes or no		yes
DC Mode			
Ch 1 Potential	-2000 to +2000	mV	50 mV
Ch 1 Filter Constant	0.2, 0.5, 1.0, 2.0, 5.0, 10.0	sec	5.0 sec
Ch 1 Gain Range	10 pA to 1 mA in steps of 1, 2, 5, 10, 20, 50, 100, 200, 500	--	100 µA
Ch 1 Output Voltage	-1.0, -0.1, +0.1 or +1.0	V	+1 V
Ch 1 Baseline Offset	-50 to +50	%	0 %
Ch 2 Potential	-2000 to +2000	mV	50 mV
Ch 2 Filter Constant	0.2, 0.5, 1.0, 2.0, 5.0, 10.0	sec	5.0 sec
Ch 2 Gain Range	10 pA to 1 mA in steps of 1, 2, 5, 10, 20, 50, 100, 200, 500	--	100 µA
Ch 2 Output Voltage	-1.0, -0.1, +0.1 or +1.0	V	+1 V
Ch 2 Baseline Offset	-50 to +50	%	0 %
Guard Potential	-2000 to +2000	mV	0 mV
Post-Run			
Ch 1 Enable Clean Cell	yes or no		no
Ch 1 Clean Cell Potential	-2000 to +2000	mV	0 mV
Ch 1 Clean Cell Duration	1.0 to 120.0	sec	1 sec
Ch 2 Enable Clean Cell	yes or no		no
Ch 2 Clean Cell Potential	-2000 to +2000	mV	0 mV

Ch 2 Clean Cell Duration	1.0 to 120.0	sec	1 sec
Guard Enable Clean Cell	yes or no		no
Guard Clean Cell Potential	-2000 to +2000	mV	0 mV
Guard Clean Cell Duration	1.00 to 120.0	sec	1 sec
Post Clean Cell Delay *	0.00 to 10.00, but ≥ longest Duration above	min	1.00

* Provides post clean cell delay values for both channels and guard.

Pulse Mode

Acquisition Delay (AD)	50 to 495	msec	300 msec
E1 Potential	-2000 to +2000	mV	200 mV
E1 Pulse Width	AD+5 to 1000	msec	500 msec
E2 Potential	-2000 to +2000	mV	-1000 mV
E2 Pulse Width	4 to 1000	msec	10 msec
E3 Potential	-2000 to +2000	mV	600 mV
E3 Pulse Width	0 to 1000	msec	1 msec
E4 Potential	-2000 to +2000	mV	-100 mV
E4 Pulse Width	0 to 1000	msec	10 msec
Full Scale Gain Range	10 pC to 10 mC in steps of 1, 2, 5, 10, 20, 50, 100, 200, 500	--	1 μC
Signal Output Voltage	-1 or +1	V	+1 V
Baseline Offset	-50 to +50	%	0 %
Filter Constant	none, low, medium, high		none

Pre-run Parameters

Pre-equilibrate	yes or no		no
Pre-equilibrate time	0.00 to 120.00	min	1.00
Auto zero prior to run	yes or no		no
Wait for thermal organizer	yes or no		no

Thermal Organizer Parameters

Activate	yes or no		yes
Set Temp	15 to 60	C	32